THE FUNCTION OF UNIVERSITIES

IN *Nature* of June 13, 1959, an account was given of the first academic convocation of the Rockefeller Institute, New York, and in succeeding weeks the addresses given on that occasion by a number of distinguished guests and by Dr. Detlev W. Bronk, president of the Institute, were printed. These addresses have now been made available in the *Rockefeller Institute Quarterly* (3, No. 2).

Dr. Bronk points out in his address that the Rockefeller Institute, from its founding half a century ago, has been a community of scholars exploring the frontiers of natural knowledge; and its traditions of intellectual excellence and adventure have laid firm foundations for a house of learning on which five years ago it was decided to build further, so that they could welcome their young successors to this community of scholars and teach and guide them as they prepared for scholarly careers. In thus extending the scope of the Rockefeller Institute, they had thought deeply of their obligations to satisfy the traditional standards of institutions of higher learning. They hoped to create an environment in which men and women could pursue knowledge as a joyous quest which would enrich all their lives and the lives of all those about them.

Dr. D. M. Knight said that the future of the Rockefeller Institute depends on the ability of other educational institutions both to provide the Institute with its graduate fellows and to maintain the climate of learning, even for and in those who themselves would never be learned men. The great aim is to produce the educated men, and for this the process of education is no substitute. Lord Adrian spoke of the place of science in universities past and present. The success of the Rockefeller Institute outside the university framework, Lord Adrian observed, shows how little we can generalize and how much we should miss if we were tied to a single pattern. Nevertheless, it is obvious that Lord Adrian is deeply concerned about the future support and organization of fundamental research, though he did not, like Prof. S. E Toulmin in a recent broadcast talk, "The Scientist's Dilemma", suggest that the real service of the scientist is to further science itself. But Lord Adrian emphasized very clearly that fundamental scientific insights are a kind of intellectual capital, providing the foundation not just of technology but also of our whole picture of Nature.

Indeed, what stands out from the addresses given at the Rockefeller convocation is not concern as to whether too many of the outstanding scientific minds are drawn into applied science, but rather the abiding responsibility of the scientist to apply his intellect critically to the problem of understanding Nature. That is, in fact, the fundamental note in Dr. H. A. Moe's concluding address. The function of the scholar in any society, Dr. Moe asserted, is to make decisions for himself and then to stand on the side of the divide that represents truth for him.

Prof. Toulmin's view of the dangers that attend the scientist now that science has become a profession, and more especially his suggestion that scientists are becoming intellectually less adventurous, and that the universities find it difficult to keep their first-rate men, has promptly been questioned, though some support could perhaps be found for it in the inaugural lecture, "The Education of Chemists", which Prof. T. L. Cottrell delivered in the University of Edinburgh on November 20. Prof. Cottrell's lecture is particularly interesting to consider in the light of the addresses given at the Rockefeller Convocation, for although he is concerned solely with the education of chemists—who incidentally are still the most numerous class of scientists—his criticisms may well have a much wider validity.

Prof. Cottrell begins his lecture by questioning the existence of any body of reliably established information about the effects of different kinds of education on the achievements of scientists. To-day, when more than two-thirds of the income of the universities comes directly from the State, university knowledge must be judged by its conformity with traditional university studies and by its internal consistency, that is, by its academic quality, on one hand, and by its relevance to the current situation in society at large on the other hand. If the traditional interests of a university become too inflexibly established, the influence of universities on the progress of human thought and of society will diminish. On the other hand, if the university's assessment of the value of knowledge becomes simply identical with that of society, the university would have lost its right to be considered as a distinctive feature of the intellectual life of society and would become indistinguishable from a training college.

Prof. Cottrell thus starts from essentially the same view of a university and its functions as was set forth by Dr. Bronk, Lord Adrian and Dr. Moe, and on this basis he examines the question whether the distinctive intellectual characteristics of the university graduate are particularly relevant to the professional work of the chemist, and, if so, whether we do sufficient to help him acquire them. Of about 21,000 qualified chemists in Great Britain in 1956, about 64 per cent were employed in industry; the 8 per cent employed directly by the Government were engaged mainly on research and development work similar to that done in industry. About 24 per cent

were engaged in general education in schools and technical colleges, and roughly some 72 per cent of chemists were engaged in applied chemistry and about 4 per cent in academic teaching and research in the universities; the activities of all the others are largely determined by what these two groups do. Further, since 80 per cent of all qualified scientists and engineers engaged in research and development are employed in establishments with a research and development staff of more than fifty, and in the chemical industry there is on average a supporting staff of 1·5 persons for every chemist, we have a picture of the average chemistry graduate engaged in research and development, not necessarily on the laboratory scale, with skilled help to carry out much of the manipulative work and with specialist services, such as analysis, provided.

Prof. Cottrell says that his personal experience gave him the main impression that the most striking characteristic of the chemist in industry is his lack of professional competence. A high proportion of honours university graduates in chemistry, he asserted, are not very good chemists, and their deficiency can be at least partly remedied by improving their education. Specifically, Prof. Cottrell says the three common defects are lack of power to apply theory, lack of a critical approach to the problem in hand and the inability to communicate clearly. Prof. Cottrell points out how costly all three defects can be, and all are aspects of the lack of an academic approach. The academic approach to knowledge is critical and theoretical and essentially concerned with its communication, and the greatest practical defect of the professional chemist is a lack of academic intellectual qualities.

Prof. Cottrell agrees that industrialists often comment that newly graduated chemists are too theoretically inclined, and it is a natural result of the present course that the newly graduated chemist tends to be crammed with a selection of facts and theories which happen to be fashionable in academic circles at the time. Academic success at present does not guarantee possession of the important academic intellectual qualities Prof. Cottrell enumerates, and granted that certain anti-academic prejudices are encountered by the university teacher in his relation with his students, it should be asked whether nearly enough is done to overcome them. In particular, he believes that the chemistry degree course at present does not foster these qualities, or give them the opportunity to develop. Prof. Cottrell's main proposal, therefore, is to provide sufficient opportunity for the student to read and think for himself. Wide and deep reading in chemistry is at present only attempted by the exceptional student.

To allow this, Prof. Cottrell suggests simply a drastic reduction in the time spent in laboratory work in the chemistry course, and a significant reduction, not perhaps quite so great, in the amount of the student's time which is organized and supervised. He frankly challenges the training argument for manipulative work, which he regards as fundamentally unsound; and while the educational argument for manipulative work is valid up to a point, he suggests that it could reasonably be reduced by half, especially if we avoid wasting laboratory time through the use of inefficient apparatus. Nor does Prof. Cottrell stop there: he would equally apply the discipline of formulating the minimum amount of knowledge a graduate should have in the field of lecture instruction, and he emphasizes the importance of avoiding the temptation to fill the time thus freed completely with exercises of a different kind. Some should certainly be filled with discussions among the students themselves, but since the application of theory is best grasped by trying to apply it, he suggests that practical exercises in calculation should replace some practical manipulative exercises, while critical thought and reading might be fostered by asking students to write essays on suitable topics and then to discuss their essays.

Prof. Cottrell's address is concerned with the education of the chemist, but the detail into which he entered is important in the general context of the balance of university education and research, partly because much of what he urges has a much wider validity, and partly because it points to a possible way of resolving the difficulties which specialization has raised between the schools and the universities. He is concerned chiefly with the defects of the average graduate in chemistry; rightly he points out that we cannot legislate for genius, and we do not know how to produce—and can only hope to recognize and encourage—great creative powers when we meet them. However, when he points out, for example, that scientific advances may, by including more facts in the scope of a wider generalization, reduce rather than increase the amount of information to be memorized, he directs attention to a factor that deserves much greater attention. If the critical review of the content of science courses generally which it implies were commonly practised, the problems of university entrance might be much less acute and those of inter-disciplinary communication might also be somewhat easier.

For all his realistic and practical suggestions, Prof. Cottrell is firmly convinced that the university teacher has a duty to society to be academic. The university is supported on a scale which is only justifiable if the academic attitude has, as he believes it has, a valuable contribution to make to society. That attitude, with its insistence on theory, and its critical and intellectual approach, is distinctive and important. It is fostered by the Rockefeller Foundation in the new departure celebrated in its first academic convocation, as it is by the universities generally. If, however, it is to be worthily upheld by the universities of Britain in the expansion on which they are now entering, they must bring that attitude to bear not only on the problems which arise in the research they undertake but no less on the problems of teaching, the content of courses, and admission and communication to which Prof. Cottrell refers tentatively indeed, but realistically also, in his stimulating inaugural lecture.

GENETIC RADIATION HAZARDS

Radiation, Genes, and Man

By Prof. Bruce Wallace and Prof. Th. Dobzhansky. Pp. xii+205. (New York: Henry Holt and Company, Inc., 1959.) 3.50 dollars.

AFTER an initial free-for-all, in which soothing or alarmist prophecies were made on a minimum of evidence, the subject of radiation hazards is now settling down and accumulating solid facts, as a basis for firmer predictions. So solid, indeed, are some of the facts released by recent work on the genetic effects of radiation that several cherished beliefs lie smashed or at least splintered. At such a time, when the real complexity of the subject is just beginning to loom ahead, it is best to tread with caution and avoid dogmatic statements, especially when writing a book. For bold generalizations may be quickly overthrown, if one is unlucky. This otherwise valuable book has suffered from such a process.

"Radiation, Genes and Man" has the laudable aim of presenting the relevant facts on which the reader himself can judge the magnitude of genetic radiation hazards and can also critically assess the pronouncements of others on the subject. In order to do this, the authors have had to cover a very wide field, ranging from elementary radiation physics to fairly advanced population genetics. On the latter they speak with authority and many will find it the most useful part of the book. But in some other fields there is a tendency towards over-simplification, which may prove misleading. For example, the section on meiosis describes and illustrates it as if no recombination takes place. The use of blue eyes versus brown as an example of simple Mendelian segregation in man has its dangers too, as there are enough exceptions and complications to make some people unnecessarily puzzled, if not suspicious.

Until recently, radiation genetics was mainly concerned with the results of irradiating post-meiotic germ-cells of *Drosophila*. In man, however, as the authors point out, effects of radiation on spermatogonia and oocytes are more important; thus most work on the mouse has been concerned with these stages. Unfortunately the authors tend to ignore the fact that results obtained from irradiating sperm which have finished dividing need not apply to actively dividing cells such as spermatogonia, in which selective forces are different. They declare, for example, that neither dose-rate nor fractionation of dose has any effect on induced mutation frequency. Yet it is now firmly established (thanks mainly to the work of Russell and collaborators at Oak Ridge) that acute X-rays have a significantly higher mutagenic effect on mouse spermatogonia and oocytes than the same dose of chronic gamma-rays, though the quality of the radiation (X versus gamma) seems unimportant. First results of this work are mentioned twice by the authors but once, unfortunately, 'crossing-over' has occurred, figures of induced mutation frequency for acute and chronic radiation being given the wrong way round (p. 131).

It is now known, too, that fractionation of dose may have a marked effect in certain circumstances. Evidence on all these matters is of great interest to man, for if lower dose-rates are less mutagenic for us, then genetic hazards from medical radiology become relatively more important than previously thought, while those from an increase in background radiation due to radioactive fall-out, etc., become relatively less important. It also means that we can no longer be content with the authors' simple definition of doubling dose as "the dose of radiation required to induce the same amount of gene mutation as that which arises spontaneously without radiation". We can now see that the answer will depend on germ-cell stage irradiated, period of time over which the dose was given, fractionation perhaps and possibly also the sex of the animal and whether irradiated as a fœtus. Thus the figure is rapidly being replaced by a formula.

Despite these criticisms, the book as a whole can be recommended, for it gives a useful introduction to theoretical aspects of radiation genetics and the problems which must be solved before we have any accurate measure of the extra genetic load imposed by ionizing radiations. The authors stress the need for further research, especially into the behaviour of mutants in populations, so that we may learn more about such important factors as the extent to which mutant heterozygotes are more or less fit than normal. In their concluding survey they rightly point out the need for "value judgments" on procedures exposing persons to radiation, in which the benefits expected are weighed against the disadvantages. Using these criteria, they conclude that nuclear bomb tests should stop. The book ends with an all-too-short glossary and a bibliography.

A. G. SEARLE

THE NEW COSMOGRAPHY

The Inner Metagalaxy

By Prof. Harlow Shapley. Pp. xiii+204. (London: Oxford University Press; New Haven, Conn.: Yale University Press, 1957.) 55*s*. net.

WHEN the history of astronomy in the twentieth century comes to be written, neither the recent developments in radio astronomy nor in satellite research will hold the primary place. The most significant discovery of the century (no matter what is yet to come) has been the recognition, in the period from 1915 to 1925, that we are inhabitants of no mean universe: the Sun was then finally relegated to its now accepted place, "an awkward position near the edge of one dusty spiral", as Prof. Shapley puts it, and the Galaxy itself was seen to be but one of millions more, scattered through space as far as our largest telescope can reach.

The complexity of modern science, and the temporal proximity of the event, do not enable us to attribute to a single twentieth century Copernicus the discovery of the new "system of the world". But the author of this book is one of a very few astronomers who brought about the greatest of all revolutions in our understanding of the universe into which we are born. It was Prof. Shapley's study of the distribution of the globular clusters that first pointed accurately the direction in which the galactic centre lay, and in the decades that followed he and his colleagues at the Harvard College Observatory sketched in, with bold strokes of the brush, the essential outlines of this larger universe which will not be radically altered. The patient counting and sampling of the millions of galaxies recorded on photographs taken at the various Harvard out-

stations gave us our first picture of the spatial distribution of the galaxies, and after nearly 30 years the Shapley-Ames catalogue of the thousand brightest galaxies is still the most useful working list for the astronomer.

In his introduction the author explains carefully the scope of his book: while much modern work is included it is not a general treatise but rather a more particular account of the Harvard contributions to extragalactic studies in the era of preliminary exploration that has now ended. This approach explains what might otherwise seem rather curious omissions. Neither 'Hubble's constant' nor 'distance scale' has any place in the index or in the text, so that only the informed reader will question the reliability of the linear distances quoted throughout the book. Most of the Harvard distances were based on criteria of apparent and absolute magnitude that are now seen to be in need of considerable rethinking, and the book would have been better if the probable nature and extent of this revision had been described more fully. On the other hand the text reminds us that many of what we often regard as quite recent discoveries about the distribution and clustering of galaxies were already contained in the earlier Harvard studies, while the chapters on the Magellanic Clouds and on the structure of our own Galaxy are thought-provoking surveys from an acknowledged authority. Here as elsewhere the author's seemly prose and enlivening choice of phrase make agreeable as well as informative reading.

Prof. Shapley's many friends will read with pleasure this appraisal of the numerous contributions from the Harvard Observatory to the field of extragalactic studies: at the same time they will have to remind their students that this book is a necessary, but not sufficient, introduction to modern cosmography.

D. W. Dewhirst

THE PHILOSOPHY OF MUSEUMS

Mostly About Museums

By A. E. Parr. Pp. 112. (New York: American Museum of Natural History, 1959.) n.p.

DR. A. E. PARR, the well-known and respected director of the American Museum of Natural History, has provided the museum profession with an unusual book. For more than twenty years he has contributed his thoughts and musings on museums to many journals and now he has collected these together into narrative form under the various aspects of museum life. He has felt rightly that in so doing it is important to give the date of the original contribution in the margin so that the reader can judge its applicability or otherwise to present-day practice. Even, however, if the idea has been entirely outmoded, the book at times forms an interesting commentary on the evolution of our present-day aims and ideals.

At the outset Dr. Parr discusses the purposes and functions of a museum of natural history and rightly expresses the view that the existence of collections, however valuable, is not a sufficient justification for the maintenance of a museum. Its purpose must be to serve "an ever-widening segment of the population" and not only a few enthusiasts whose interests lie in particular directions. Its functions range from research to pure entertainment, and all its service to formal education lies between these two extremes. Dr. Parr also rightly emphasizes the distinction between formal class-room teaching and the general education of the public based on voluntary and perhaps rather casual attendance at a museum.

The author refers to the naturalism which is generally shown in habitat groups and cites experiments in using an impressionist background. He believes that breaks with complete realism should be possible and feels that such a method of display would be of particular interest to children. The probability is that such exhibits would be produced at lower cost than the one in which a strictly naturalistic scene is enforced. On the other hand, it would require more imagination on the part of the curator and at times more apprehension on the part of the public.

Dr. Parr deals with the subject of research by the staff of museums in some detail. As a man of research himself he is naturally insistent that it is a function of museums, though he feels that it is a right exercised by the individual and not one controlled by the governing authority.

This is a series of essays which should be read and re-read by all curators of museums and also by the members of their governing bodies. By apt phrase and a tremendous insight into the philosophy and real needs of museums Dr. Parr has done much to clarify the present position consequent on the rapidly changing world in which we live. F. S. Wallis

THE ST. LAWRENCE SEAWAY

The St. Lawrence Seaway

By Lionel Chevrier. Pp. x+174. (Toronto: The Macmillan Company of Canada Limited; London: Macmillan and Co., Ltd., 1959.) 21*s*. net.

The St. Lawrence Seaway

By T. L. Hills. Pp. 157. (London: Methuen and Co., Ltd., 1959.) 12*s*. 6*d*. net.

THE St. Lawrence Seaway, officially opened in June 1959, has been rightly acclaimed as a magnificent achievement in the fields of engineering and of international co-operation. After the spate of magazine articles and films that accompanied its opening it is understandable that there should now be appearing several authoritative and analytical books on the Seaway. The two reviewed here differ greatly in approach, objective and context. Mr. Lionel Chevrier's account is that of one who has been intensely interested in the project for many years and who was closely associated with it after 1945, first as Canada's Minister of Transport and then as president of the St. Lawrence Seaway until 1957; he claims to have been "in a particularly good position to see what was happening, to follow the events from day to day, and to judge of these as they came across my desk". Some readers will feel that he was too close to the scheme to give perspective and objectivity to his story. Such will find the book by Prof. T. L. Hills, of McGill University, a useful corrective, for it is an account of the whole scheme in its wider setting such as one would expect from a geographer, with a balanced account of the development of the idea of the Seaway and its associated power projects and of their later fulfilment, followed by a shrewd assessment of the future prospects.

The engineers' triumph has been tremendous—"one of the most ambitious and effective man-made alterations to the face of the earth ever completed", Mr. Chevrier calls it. It has created a navigable waterway, 2,000 miles in length and 27 ft. in depth, right into the heart of North America, and open to the shipping of many nations. It will be especially used for the transport of iron ore from Quebec and Labrador to the Great Lakes and of grain from the Lakes to ports on the lower St. Lawrence. For the large amount of electrical power made available, there existed a huge demand in both southern Ontario and in upper New York State even before constructional work began. Whether the Seaway will be as fully used as it needs to be to make it an economic proposition remains to be seen. Mr. Chevrier, seeing the Seaway as "one of the major continuing themes of Canadian development", needs no convincing. "The St. Lawrence Seaway showed us", he writes, "that, almost without knowing it, we had become a great power". Prof. Hills is more cautious, and shows how, in the economic field, the claims for the Seaway have ranged from unbridled optimism to complete pessimism. He faces many of the problems fairly and objectively and on several issues cautiously pronounces that "only the future will tell".

The traffic figures for the early months of the Seaway have, it seems, been disappointing. It is therefore essential that right at the outset careful attention should be directed towards any possible new developments and to the potential bottlenecks and difficulties that could create both economic and political problems. These two books, and especially Prof. Hills's, throw light on these possibilities and give valuable insight into the inception and execution of a great scheme—as imaginative in many ways as that of the Tennessee Valley Authority, and politically one fraught with even greater difficulties because of the international complications. Whether the Seaway comes to be reckoned a complete success, economically and politically, must turn on the extent to which it proves, as a public utility, to be "self-liquidating within a reasonable period"—the aim of the hard-headed American negotiators from the very beginning.

R. W. STEEL

TEXT-BOOK OF BACTERIOLOGY

A Text-Book of Bacteriology

By Dr. R. W. Fairbrother. Eighth edition. Pp. viii+502+7 plates. (London: William Heinemann (Medical Books), Ltd., 1959.) 25*s*. net.

An Introduction to Bacterial Physiology

By E. L. Oginsky and Wayne W. Umbreit. Second edition. Pp. xii+443. (San Francisco and London: W. H. Freeman and Company, 1959.) 7.50 dollars; 54*s*.

A Guide to the History of Bacteriology

By Prof. Thomas H. Grainger, Jr. (Chronica Botanica: an International Biological and Agricultural Series, No. 18.) Pp. xi+210. (New York: The Ronald Press Company, 1958.) 4.50 dollars.

FAIRBROTHER'S "Text-book of Bacteriology" has been popular with medical students since its first appearance just before the Second World War. It is an extremely well-laid-out book, opening with general bacteriology and then passing on to systematic bacteriology and virology. General techniques, staining, etc., are given in the final section. It is very readable and of a convenient size.

It is, of course, possible to find fault with almost any book by a painstaking review of its contents, but, nevertheless, there are a few features of this book which are weak and should be strengthened in the next edition. The whole section devoted to sterilization is weak; for example, on p. 58, "X-rays and radium appear to have no marked bactericidal action", while the portion devoted to the autoclave has no mention of the many important precautions to be taken in using this machine. The importance of methods of sterilization is being more widely appreciated in medicine, and the student should be able to get enough information from the only text-book on the subject which he is likely to buy.

In the systematic section the discussion on the use of staphylococcal phage typing is very short, while on p. 247 the alcohol-killed and preserved typhoid vaccine is recommended. The space devoted to virus infections is short and some of the ideas, for example, the spread of poliomyelitis virus along the olfactory nerves, are not generally held.

Having made these adverse comments, the reviewer is left to say that it is, nevertheless, a good text-book which may be safely recommended to medical students.

"Bacterial Physiology" is obviously set to become as popular in its field as Fairbrother is with students. There may be some doubts in Great Britain as to the exact audience for which this book is intended. While it is obviously compulsory reading for microbiologists and applied chemists, need the medical bacteriologist enter these deep waters? Before passing an opinion on this point it may be as well to review the scope of the work. "Bacterial Physiology seeks to determine what happens in bacteria, how it happens and why it happens". After this definition, the authors cover bacterial anatomy, problems in bacterial growth and population control, metabolism, a miscellaneous group including photosynthetic bacteria and phage and finishes with a consideration of the capacity of the cell, for example, adaptation and virulence.

The text is quite self-contained in that no prior knowledge of biochemistry is needed to understand the processes described. The whole text is enlivened by a series of attractive and instructive drawings. This is, in short, an ideal book to guide the medical bacteriologist into an understanding of the tremendous strides that are being made into the fundamentals of his subject and can be unhesitatingly recommended.

"A Guide to the History of Bacteriology" is in some ways a misleading title as it is, in fact, an annotated bibliography divided into four sections. It opens with a selective guide to the literature and then goes on to the history of bacteriology, where it reviews each topic in turn, for example anaerobic bacteria. The third section is devoted to general biographical references and the last to a selective guide on the biographies of individual bacteriologists.

It is obviously a difficult book to evaluate without using it in practice and the reviewer is unable to decide its value to specialists in the field. For the general worker it appears to offer a number of useful starting points for a search in the literature on any specific topic. At the same time it is a mine of interesting information; for example, Section 88 devoted to "Age of Bacteriologists for Best Work" should provide material for many a laboratory discussion.

R. H. GORRILL

RADAR 'RING ANGELS' AND THE ROOSTING MOVEMENTS OF STARLINGS

By E. EASTWOOD, G. A. ISTED and G. C. RIDER
Research Division, Marconi's Wireless Telegraph Co., Ltd., Great Baddow, Essex

RADAR observations upon the ring angels reported by Eastwood, Bell and Phelp[1] have been continued throughout 1959. Analysis of the film records obtained has yielded a total of approximately three hundred such incidents and the geographical distribution of the active centres is shown on Fig. 1. The dots upon this map mark the centres of ring angels which have been observed on one occasion only. The small circles show the positions of ring sources which have been observed to be active on several occasions; the number of observed events is shown within the circle.

As the number of observations accumulated, it became possible to pin-point the centres of the persistent ring angel sources more accurately; further investigation established that starling roosts were situated at these centres. A ring source of special interest is that located within the London region; it is roughly centred upon Trafalgar Square and must arise from dispersal of the starlings which roost upon the buildings grouped about the square. This observation was rendered possible by the Doppler device, which discriminates between a moving target and the fixed ground echoes.

The fact that starlings leave a roost about sunrise, in discrete flocks separated at intervals of a few minutes, has long been known to ornithologists; but the manner of the subsequent dispersal of the birds could not be precisely determined by ordinary bird-watching methods. By the use of radar Ligda[2] has shown that masses of red-winged blackbirds, flying out from a common roosting ground at Texarkana, show up on the plan position indicator as an expanding single diffuse ring. Harper[3] has reported radar echoes in the form of expanding arcs of circles and has attributed them to the movement of starlings from a roost located six miles from his radar station at East Hill, Hertfordshire. Echoes in the form of curved lines moving seawards have been described by Lack[4] and associated by him with starling flocks migrating direct from roosts.

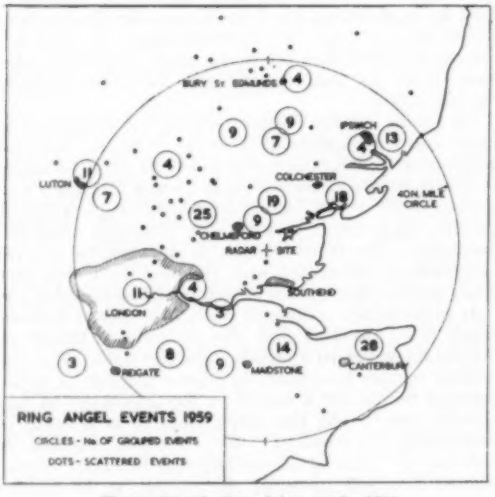

Fig. 1. Distribution of ring angels, 1959

In the present series of radar experiments observers, equipped with radio-telephones, were located at a number of ring angel sources selected from Fig. 1. These observers signalled over the radio link to the radar station at the precise moments of emergence from the associated roost of the successive flocks of starlings. These signals were impressed upon the film which was recording the radar plan position indicator picture. Subsequent projection of the film establishes perfect correlation between the emergence of the successive rings from a point source on the plan position indicator and the eruption of the birds from the roost. In some incidents the echoes are of almost uniform amplitude around the ring, indicating uniform distribution of the birds; in other cases incomplete circles show preferred directions of flight, but this pattern is not constant for a given roost. Fig. 2 shows a spectacular display of ring angels.

This radar experiment clearly establishes that flocks of starlings emerging from a roost may afterwards take the form of a series of expanding, concentric rings of birds flying radially outwards from the roost. It follows that the ring angel source should remain fixed and independent of the wind; this we have confirmed. It also follows that the expanding ring of birds will suffer displacement by the wind relative to the point of emergence. This is shown on Fig. 2 where the Canterbury ring (33 miles to south-east) shows a displacement to the south-west by an amount corresponding to the wind speed.

The manner of assembly of the birds into the roost is scarcely less remarkable than the ring dispersal although markedly different in form. Fig. 3 shows the radar record of the evening assembly in progress at Matching Green, Essex; the birds are extended over a funnel of five miles and are approaching from the south-west. As noted by Witherby[5] the progress of birds towards the roost normally terminates at a pre-assembly point near to the roost and this process may take as long as an hour. Our observations upon the Essex roosts show that the final entry into the roost normally takes place about sunset and is completed within a few minutes. The entry may take the form of a sequence of departures from the pre-assembly region by discrete flocks of birds at intervals of about half a minute; these flocks fly straight into the roost. This disciplined behaviour of final entry is obviously related to the ring dispersal

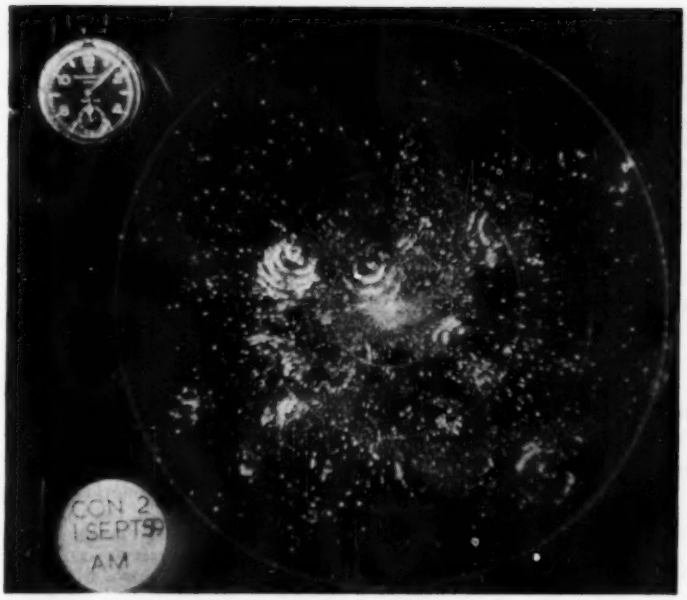

Fig. 2. Display of ring angels, sunrise, September 1, 1959

phenomenon. Even more spectacular is the continuous entry from a vast flock of birds circling the roost.

The pre-assembly process can only be studied by radar and shows wide variation in form, with some dependence upon the direction and speed of the wind. The assembly pattern shown on Fig. 3 does not happen every evening and sometimes the assembly process is quite formless with a general dribbling in of flocks of birds from all directions—which is what would be expected from the nature of the morning dispersal. The Fig. 3 type of approach, on the other hand, demands that the birds must perform a circular movement during the day in order to bring them into the approach funnel. This operation, and the circumstances that provoke it, remain obscure.

The essential feature of the ring dispersal phenomenon is the pulsed departure of the birds in successive groups, the rings being the consequence of the constant headings flown by the birds. This periodic eruption of the birds from the roost possesses some of the features of a relaxation oscillation and suggests an excited, emotional state of the birds. We have recorded the noise-level of the starling song from the roost, using a 4-ft. paraboloidal mirror fitted with a microphone at its focus. The total noise emitted by the roost decreases with the departure of the successive flocks; but the most striking feature of the noise record is the sudden quenching which occurs at the moment of departure of each of the waves; an abrupt diminution of noise-level by as much as 10 decibels may then occur which is rapidly restored after the departure of the group.

The roost organization and flight behaviour appear all the more remarkable when it is remembered that such communities may contain hundreds of thousands of birds. We have estimated the minimum population of the Matching Green roost in Essex by assessing the density and area of field covered by the birds when they are following the field mode of pre-assembly. This population figure has agreed in order of magnitude with that derived from radar measurement of the echo amplitude of the rings. In this calculation we have taken the radar cross-section of a starling as $2{\cdot}5 \times 10^{-3}$ sq. m., as suggested by Edwards and Houghton[6].

Analysis of the time variation of ring angel incidents observed from the Essex radar site during 1959 shows a maximum during July–August; no ring dispersals were observed during the four weeks mid-April–mid-May; but some activity was recorded for all other weeks of the year. These results may be related to the breeding habits of the starling but it must be remembered that the frequency of occurrence

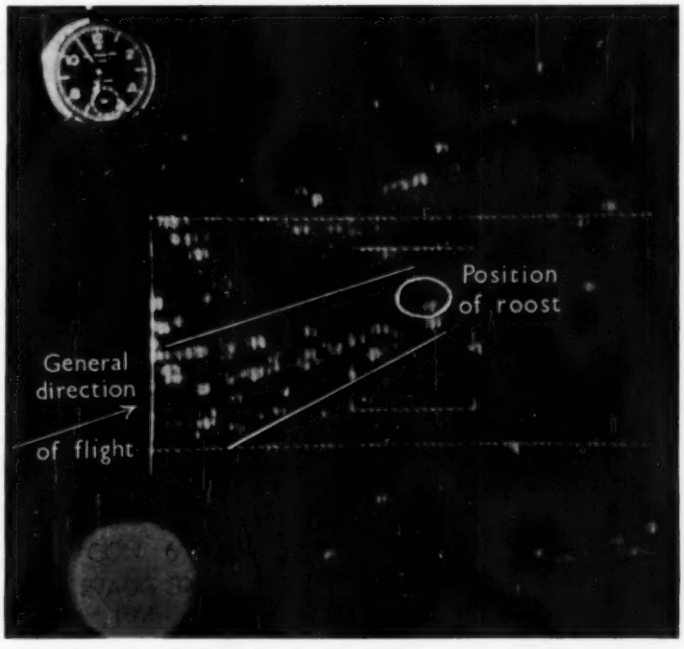

Fig. 3. Starling assembly, Matching Green, Essex, August 27, 1959
(Magnified portion of plan position indicator)

of ring dispersals, while related to the number of active roosts, is also dependent upon the roost organization, flight behaviour and feeding habits of the birds, and these factors may also be subject to seasonal variation. Having regard to the number of permanent roosts, and also the presence of temporary roosts of immigrants during the autumn and winter, it is in fact rather strange that the ring dispersals do not occur much more frequently.

We wish to thank our colleagues of the Radar, Propagation and Photographic Groups who have made the observations possible.

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TREATMENT OF DEPRESSION BY DRUGS AND OTHER MEANS

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DEPRESSIVE illnesses are disorders of great social and medical importance by virtue of their prevalence in the community and the frequency with which they are responsible for morbidity, loss of efficiency and working capacity, admission to hospitals and for loss of life by suicide.

During the past two years new drugs have been introduced which are claimed to be highly effective for the treatment of depressive illnesses. These claims merit serious consideration in view of the potential importance of any treatment which will result in alleviation or recovery in such socially important disorders.

This article presents a critical review of the various drugs which are used for treating depression and an appraisal of their place in relation to other current therapeutic methods.

First of all, it will be desirable to describe briefly the main clinical features of depressive illness in order to facilitate consideration of the therapeutic and clinical effects of the drugs and other methods for treating depression. The term 'depression' is used clinically in two distinct senses. It is used to denote a symptom which can occur in many forms of psychiatric and physical illness and also to illnesses in which the predominant feature is a characteristic mood change variously referred to as depressive illness, depressive state, depressive disorder and sometimes as melancholia.

Depressive illness has a characteristic syndrome of symptoms and signs consisting of change in mood, difficulty in thinking, behavioural changes and physiological disturbances.

The change in mood is relatively fixed and persistent and may vary in severity in different individuals, from mild despondency to extreme and abject despair. The change in mood is accompanied by difficulty in thinking and making decisions, by preoccupation with painful thoughts, and by decrease in interest, energy and initiative. Physical complaints in depressive illness are varied and include loss of appetite and weight, decrease or complete loss of sexual libido, lack of energy, dyspepsia, bodily pains, etc.

Depressive illnesses are the resultant of the interaction of genetic and constitutional factors on one hand with environmental and other exogenous influences on the other. Those depressive illnesses which are predominantly determined by genetic–constitutional factors are usually referred to as endogenous depressions and are characterized by a typical diurnal variation, being worse in the morning and tending to improve later in the day, and an onset which may occur independently of adverse environmental circumstances. Depressive illnesses which are predominantly a reaction to environmental influences are referred to as reactive depressions and are usually less severe and often fluctuate in severity according to external circumstances.

In a large number of depressive illnesses, however, both endogenous and environmental factors are present. In clinical practice an infinite variety of combinations of these factors are found as shown diagrammatically in Fig. 1.

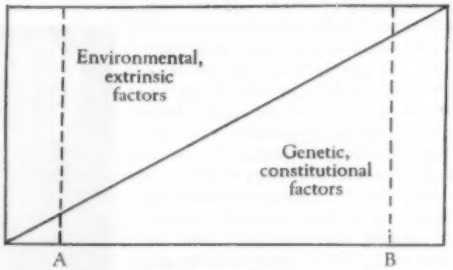


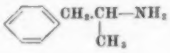
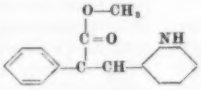
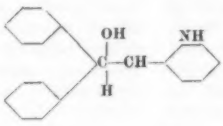
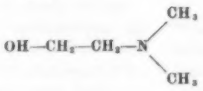
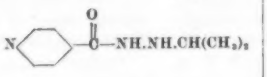
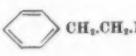
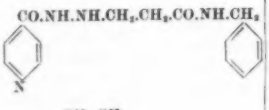
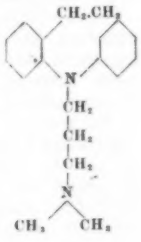
Fig. 1. *A*, representing patient with predominantly reactive depression; *B*, a patient with predominantly endogenous depression

The history of the medical treatment of depressive states extends over two thousand years. Hippocrates in the fourth century B.C. provided a detailed clinical description of depressive illness. The term melancholia has its derivation from the humoral theories of disease causation propounded by Hippocrates and Galen, who considered the illness to be caused by an excess of black bile.

Hippocrates used herbal remedies for depression such as black hellebore, borage, bugloss, marigold and epithyme. These remedies continued to be popular for many centuries and were advocated by Burton in his classical treatise "Anatomy of Melancholy", and as pointed out by Marley[1] were still in vogue in the mid-seventeenth century.

The modern era of pharmacotherapy of depression virtually began with the clinical use of the amphetamine drugs some thirty years ago. A large number of

Table 1

Generic name of drug	Chemical constitution	Chemical formula	Usual daily dosage-range (mgm.)	Side-effects, complications, disadvantages, toxic actions
Amphetamine	β-phenyliso-propylamine		5–20	Sympathomimetic actions produce side-effects. Disadvantage in producing post-medication fatigue and depression. Danger of addiction and psychotic episodes.
Methylphenidate	Methyl phenidyl acetate hydrochloride		5–20	Sometimes accentuates anxiety symptoms, schizophrenic manifestations and hysterical symptoms.
Pipradol	α-2-(piperidyl) benzhydrol hydrochloride		5–25	Anxiety, agitation, delusions and hallucinations are sometimes made worse.
Deanol	2-dimethyl amino-ethanol		10–50	High dosage may produce insomnia, increased muscular tension and isolated muscle twitches.
Iproniazid	*l-isonicotinyl-2-isopropyl* hydrazine phosphate		75–150	Hypotension with palpitations, dizziness and syncope on postural change. Œdema, impotence and toxic liver necrosis. Hypomanic and manic excitement. Development of schizophrenic and paranoid features.
Phenelzine	β-phenyl-methyl hydrazine		45–90	Side-effects are infrequent; include hypotension, nausea, vomiting, drowsiness, headaches, impotence, œdema, constipation. No toxic effects reported.
Nialamide	*N-isonicotinoyl N'-(β-N-benzyl carboxamido-ethyl)* hydrazine		25–150	Increase of anxiety and agitation. No toxic effects reported.
Imipramine	N-(γ-dimethyl-amiaopropyl)-iminodiben yl hydrochloride		50–250 according to stage of treatment	Dryness of mucous membrane. Excessive sweating, gastro-intestinal symptoms, dizziness, palpitations, disturbance in visual accommodation. Allergic state, photosensitization, urticaria, angioneurotic œdema and pruritus. Tremors and Parkinsonism.

central nervous system stimulants and antidepression drugs have since been introduced, the main ones being shown in Table 1, which gives information on chemical composition, dosage, side-effects, disadvantages and dangers.

Amphetamines and Related Drugs

Amphetamine was first used for treating depression by Peoples and Guttman[2] and Myerson[3]. Amphetamine in oral doses of 5–15 mgm. usually produces increased alertness, elevation of mood, euphoria, an increase in psychomotor activity and initiative with relief of fatigue. Similar effects are produced, in smaller doses, by dextroamphetamine (the dextro isomer of amphetamine) and methamphetamine, its methyl derivative.

However, there is considerable individual variation in response to amphetamine drugs. Some persons, instead of experiencing an elevation of mood and an increase in well-being, become anxious, agitated or depressed. Amphetamines also have unpleasant side-effects arising from their sympathomimetic actions, namely, dryness of mucous membranes, loss of appetite, increased heart-rate with symptoms of palpitations, etc. Further disadvantages are the transience of the elevation in mood which is usually followed by increased feelings of depression, irritability and fatigue, and the fact that tolerance to amphetamine rapidly develops with the result that dosage has to be increased in order to produce the same effects. Addiction to amphetamine is not uncommon and the drug in high doses may induce psychotic states as described by Connell[4].

Phenmetrazine (preludin) is an oxazine derivative similar in chemical structure to amphetamine and has sympathomimetic effects. It is used to reduce

appetite for the treatment of obesity and sometimes for its euphoriant effects. Phenmetrazine until recently could be bought without medical prescription, and misuse and addiction to the drug has been described by Bethel[5] and Evans[6], who reported that preludin sometimes produced severe psychotic states.

Methylphenidate

Investigations by Meier *et al.*[7] on animals and by Heiss *et al.*[8] on human volunteers revealed methylphenidate to be a central nervous system stimulant producing an increase in psychomotor activity. Favourable results were reported in the treatment of depressive states with the drug by Stier[9], Geller[10], Jacobson[11], Natenshon[12], Davidoff *et al.*[13] and Pennington[14]. Plummer *et al.*[15] showed that methylphenidate had a specific antagonistic action to reserpine and it was used clinically with success for treating the depression and other side-effects of reserpine therapy by Ferguson[16], Carter[17], and Madi and Kovitz[18]. Recent studies, however, have attracted attention to the limitations, dangers and disadvantages of the drug. Bartlett[19], for example, found that anxiety symptoms, schizophrenic manifestations and hysterical symptoms were made worse by the drug, and Robin and Wiseberg[20] in a double blind controlled study found no evidence that methylphenidate had significantly better effects in relieving depression than that associated with the administration of inert tablets.

Pipradol

Pipradol differs from the majority of other antidepressant drugs in not being sympathomimetic. Brown and Werner[21] reported that pipradol produced co-ordinated hyperactivity in animals without evidence of post-medication depression.

Early clinical trials of pipradol in depressive states were carried out by Fabing[22], who reported that both reactive and endogenous depressions responded satisfactorily. Fullerton[23] found the drug most helpful in chronic reactive depression, but that anxiety, agitation, delusions and hallucinations were made worse and their presence constituted contraindications to the use of the drug.

Begg and Reid[24] found pipradol to be of no value for treating endogenous depressions and which sometimes even became worse. Reactive depressions sometimes benefited, but this was offset by the risk of increasing anxiety and sometimes of precipitating psychotic episodes.

The available evidence, therefore, indicates that neither methylphenidate nor pipradol are of significant value for treating depressive states.

Deanol

Deanol (2-diethylaminoethanol) is one of the recently introduced drugs for treating depression. It was reported by Konigsmark, Killam and Killam[25] to have a weak stimulating effect on the electroencephalogram and by Pfeiffer and Murphee[26] in normal volunteers to produce increased powers of concentration, elevation of mood and wakefulness, while its stimulating effects lasted from 34 to 48 hr. without any post-medication depression, fatigue or irritability.

Lemere and Lasater[27] in a preliminary report of a clinical trial with deanol reported that it was effective in relieving symptoms of fatigue and depression. Quite recently, reports from controlled studies in Great Britain have failed to confirm the claims made by American workers and the drug has now been withdrawn in Great Britain.

Iproniazid

Iproniazid, discovered by Fox and Gibas[28], was first used for treating tuberculosis. Although effectively promoting the healing of tuberculosis lesions as well as producing general clinical improvement, the drug had to be discontinued because of its undesirable side-effects. Some of the untoward side-effects were due to stimulation of the central nervous system which produced elevation of mood, disturbed overactive behaviour and sometimes psychotic episodes. Iproniazid was first tried in chronic mental disorders by Crane[29], who reported only limited beneficial results.

Subsequent reports of clinical trials with iproniazid in depressive states vary greatly both regarding recovery and improvement rates as well as the types of depression reported to benefit. The recovery and improvement rates, reported by different authors, varies from 75 per cent by Robie[30] and Kline[31] to 30 per cent by Settel[32].

Dally[33] states that mild depressive states with loss of weight respond best, whereas Alexander[34] found that inert, apathetic types of depression which had not responded to electroplexy were helped most. Delay and Buisson[35], on the other hand, found that mild depressive states responded best, whereas West and Dally[36] reported that atypical depressive illnesses were most susceptible to treatment by iproniazid.

As these reports are from uncontrolled trials, it is not surprising that such discrepancies occur in view of the many sources of error, which can only be effectively dealt with by appropriate central procedures.

The enthusiastic reports of Kline[31] and Robie[30] regarding the beneficial results of the drug in depressive states even in long-standing cases aroused great interest and stimulated biochemical and pharmacological research into the actions and effects of the drug.

Iproniazid was first shown to inhibit monoamine oxidase by Zeller *et al.*[37]. Undenfreund *et al.*[38] demonstrated that iproniazid produced a rise in the level of brain serotonin. This finding appeared to be of particular significance in view of the fact that reserpine, which not infrequently induces severe depression, causes a decrease in the level of brain serotonin. The postulated relations between therapeutic action in relieving depression and monoamine oxidase inhibition, although forming an attractive hypothesis, cannot be fully accepted for reasons given below.

In a fully controlled double blind study of iproniazid in depressive states, Rees and Benaim[39] found that only 20 per cent of depressed patients showed either moderate or marked improvement with the drug. The improvement with iproniazid, however, was statistically significantly greater than that associated with the control procedure. Reactive depressions responded best, next endogenous depressions and atypical depressions worst. Eighteen patients who derived no significant benefit from iproniazid were given electroplexy; of these, sixteen responded immediately, making a complete recovery, one patient improved but did not recover fully and only one patient failed to improve. The response to

electroplexy was so striking in speed as well as in the degree and quality of recovery that there was no possible room for doubt regarding the supremacy of electroplexy over iproniazid in these patients.

Rees and Benaim[39,40] reported a number of undesirable side-effects resulting from iproniazid, for example, œdema, symptoms due to hypotension such as palpitations, dizziness or syncopy on postural change. Hypomania developed in three patients. Some patients with atypical depressive states developed paranoid ideas and a schizophrenic type of thought disorder. Two patients who developed jaundice are described in detail elsewhere[41]. The most serious toxic effect of iproniazid is liver necrosis leading to jaundice, sometimes with a fatal outcome as reported by De Verteuil and Lehmann[43], Fisher and Olver[45], Pare and Sandler[44], Smarr *et al.*[45], Weiss *et al.*[46], Zetzel and Kaplan[47], Frantz[48], Shay and Sun[49]. In view of the danger of these serious toxic effects and its limited therapeutic effects, Rees and Benaim[40] conclude that the use of iproniazid for treating depressive states is no longer justified. Research on other monoamine-inhibiting drugs has yielded two new anti-depressant drugs which have recently been released for clinical trial in Great Britain, namely, phenelzine and nialamide.

Phenelzine

Phenelzine is a hydrazine derivative, a sympathomimetic amine, having potent inhibiting actions on monoamine oxidase. Phenelzine is claimed to be a more potent monoamine oxidase inhibitor than iproniazid and that it produces its therapeutic effects more quickly and which are more lasting. A number of reports of clinical trials with phenelzine have appeared from the United States; for example, Thal[50] found that it was a more potent anti-depressant than ten other remedies for depression. He states that an elevation in mood and an increase in energy usually occurred within 48 hr. and that gradually patients became more active and alert, slept better, improved in appetite and gained in weight. Favourable reports on its actions in depressive states are also given by Saïnz[51], Goldman[52], Furst[53] and Saunders[54]. Side-effects, which were relatively infrequent, include nausea, vomiting, drowsiness, headaches, transient impotence, ataxia, œdema and constipation. No toxic effects on the blood, kidneys or liver have been reported. A double blind controlled study of phenelzine in the treatment of depressive states was carried out by Rees and Davies[55], who found that phenelzine produced significantly greater improvement than inert tablets. It was mainly effective in mild depressive states and was only of limited value in severe endogenous depressive states. A number of patients who did not show significant improvement with phenelzine were given electroplexy, which produced such rapid and marked improvement that its supremacy over phenelzine was unequivocal.

Nialamide

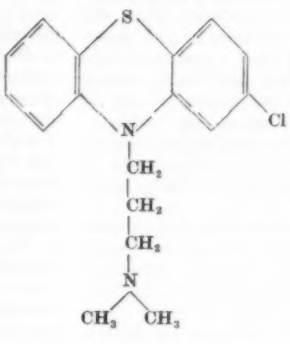
Nialamide is another new anti-depressant drug also claimed to be a more potent amine oxidase inhibitor than iproniazid and alleged to be free from its toxic effects. Trials with this drug are proceeding in Great Britain and other parts of the world; but it is as yet too early to assess accurately its therapeutic value in depression.

Dewhurst and Pare[56] tried the drug in 28 depressed patients and confirmed that it was not toxic. Nine improved and when given inert tablets 3 continued to improve, 3 relapsed and improved again when re-started on the drug and 3 are still under investigation. A serotonin tolerance test showed unequivocal inhibition of amino-oxidase in 10 patients who showed no clinical improvement. Feldstein *et al.*[57] showed that 1-benzyl-2-methyl-5-methoxytryptamin is an effective monoamine oxidase inhibitor in man but has sedative rather than stimulating properties. Furthermore, it is also difficult to relate the therapeutic effects of monoamine oxidase inhibiting anti-depressant drugs to the rate of inactivation of catechol amines, as the metabolism of adrenaline and noradrenaline involves methylation giving rise to pharmacologically inactive 3-methoxy derivatives before oxidative deamination by monoamine oxidase occurs.

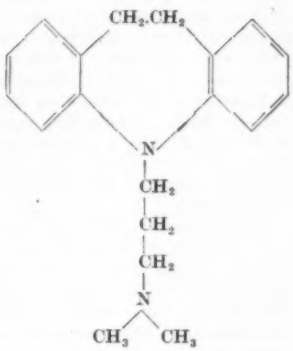
Imipramine

Imipramine is an iminodibenzyl derivative, somewhat similar in chemical structure to chlorpromazine as shown below :

Chlorpromazine



Imipramine



It differs from the majority of anti-depressant drugs in being neither a central nervous system stimulant nor an inhibitor of amine oxidase. Sigg[58] showed that imipramine produced sedation in experimental animals and in contrast to cerebral stimulants and amine oxidase inhibitors did not counteract the sedative effect of reserpine. The animal studies of Van Meter, Owens and Himwich[59] on the electrophysiological effects of imipramine showed that it increased the excitability of the brain and raised serotonin and catecholamine brain levels, but to a lesser extent than iproniazid.

Imipramine is a complex drug which influences many brain mechanisms, stimulating some and depressing others. In low doses it blocks the reticular formation, evoking a sleep brain wave pattern and preventing the alerting response in the electroencephalograph, thus being similar in this respect to chlorpromazine. Imipramine was first investigated clinically by Kuhn, who found that it had only limited clinically beneficial effects, but on re-investigating some years later Kuhn[60] found that it was therapeutically effective in depressive states. Kuhn reported improvement in 75 per cent of a group of 40 depressed patients treated with imipramine. Kielholtz

and Battegay[61] found that imipramine improved 50 per cent of a group of depressed out-patients and 67 per cent of a group of 69 depressed in-patients.

Lehmann, Kahn and De Verteuil[63] in a trial of imipramine on a group of 84 patients suffering from depression found that 30 per cent showed marked improvement or full recovery within two weeks and that the figure rose to 60 per cent after eight weeks of treatment. Fazio, Gilberti and Loeb[63] reported that 74 per cent of their group of depressed patients recovered or improved with the drug. Azima and Vispo[64] found that imipramine specifically relieved depression but did not improve anxiety. Saucier and Dumas[65] report that manic depressive illnesses and involutional melancholia improved with imipramine in a third of the cases, but that neurotic depressive patients did not benefit. Hoff[66] found imipramine to be most helpful in endogenous depression and for the mood fluctuations following electroplexy, but was ineffective in chronic depressions. Delay and Deniker[67] found that beneficial results were obtained in about 50 per cent of depressive patients who would be deemed suitable for electroplexy. They recommend its main use should be for slight or moderate depression.

Very few fully controlled studies have been reported on imipramine in the treatment of depressive states. A double blind fully controlled trial of imipramine in depressive states carried out by Rees, Brown and Benaim[68] provided evidence that the drug produced significant improvement in some features of depressive states but that it was relatively ineffective in severe endogenous depressive states. A number of patients who had not improved significantly with imipramine were given electroplexy, which resulted in an immediate marked improvement. The evidence clearly indicated that imipramine is far less effective in severe depressive states than electroplexy.

A recent controlled trial has been reported by Ball and Kiloh[69], who found that the drug gave significantly better results than the control procedure using inert tablets both in endogenous and reactive depressions and that it was more effective than iproniazid in the treatment of endogenous depression.

Imipramine frequently has unpleasant side-effects such as dryness of the mucous membranes, excessive sweating, gastro-intestinal symptoms, dizziness, palpitations, disturbance of accommodation, allergic reactions, including photosensitization, urticaria, angioneurotic œdema and pruritus. In some patients tremors and mild Parkinsonism occur and occasionally states of excitement have been reported.

Designs of Drug Trials

The majority of clinical reports on the new antidepressant drugs, unfortunately, are based on uncontrolled studies which permit few or no valid conclusions to be made from the results. Failure to employ appropriate controls has delayed the scientific evaluation of anti-depressant drugs. It is interesting to note that the rate of improvement or recovery reported from double blind controlled trials is usually in the order of 20–30 per cent, whereas in uncontrolled studies it is 60–85 per cent. The assumption is only too frequently made that any clinical improvement occurring when a drug is being administered is necessarily attributable to its pharmacological properties. This assumption is, of course, quite unwarranted as the course of a depressive illness can be influenced by many factors within the patient or in his environment. Furthermore, a depressive illness often shows fluctuations and usually has a tendency to have a self-limiting course. These are only some of many factors which can vitiate the results of uncontrolled studies.

The possible therapeutic effects of the trial procedure itself due to suggestion or the increased medical and nursing care, interest and attention, are also sources of error which need to be assessed and controlled.

Errors may also arise from heterogeneity of the clinical material comprising the group investigated, from biased allocation of the active treatment and the control procedure to patients; from unequal application of criteria of improvement to the actively treated and control groups and finally from bias or anticipation on the part of observer and patient when the trial is not carried out under 'blind' conditions.

The complexity of psychiatric material makes it virtually impossible to match accurately patients either into pairs or into groups for comparing the effects of active treatment and the control procedure. The method whereby the patient acts as his own control overcomes the difficulty of matching patients and is particularly useful for drug trials in psychiatry. This method was used by me and my collaborators in controlled clinical trials of iproniazid, phenelzine and imipramine in depressive states and is described briefly as an example of one type of trial design.

Before starting the trial, the clinical and diagnostic criteria for inclusion of patients in the trial were agreed and laid down. Clinical status was assessed by detailed methods, including ratings of a large number of clinical features and items on both objective features as well as subjective symptoms. Special record sheets were designed for the investigation, and criteria to be used in recording clinical state and progress were set down in detail beforehand.

The active drug and the corresponding inert tablet, which was identical in colour, size and appearance, were administered in exactly the same way for three weekly periods at a time. The allocation of the active drug and the inert preparation for the first three-week period was achieved by a randomizing procedure to avoid possibility of bias and to ensure the probability that approximately half the group would start with the active preparation and the other half with the inert preparation. For the second three-week period those who started with active tablets were then given inert tablets and vice versa. Thus active and inert tablets were then given for three-weekly periods in alternation, for a total period of twelve weeks for the iproniazid investigation and a total of six weeks for the phenelzine and imipramine studies. The random allocation of drug and inert tablets for the initial period and subsequent alternation of active and inert tablet periods equalizes the chances of the manifold factors, other than the pharmacological action of the drug, operating during the period of administration of the active drug, as during the period of inert tablet administration strictly blind controlled conditions were observed. The batches of tablets were identified by series of random numbers; the only person knowing their identity was the dispenser. As an additional precaution the results were fully assessed statistically before the code was broken, thus making the trial 'treble blind'. The method of sequence control (Rees and Lambert[70]) also permits any 'carry-over'

effect of the active drug into the ensuing inert tablet period to be detected and assessed.

Place of Anti-depressant Drugs in Relation to Other Methods of treating Depressive States

It is convenient to consider the treatment of mild depressive states separately from that of severe depressive states. Mild depressive states, which often have a tendency to become chronic, are mainly treated by social, psychological and symptomatic measures. Social treatment usually takes the form of manipulation of conditions at home or at work. Social treatment can be effectively combined with supportive psychotherapy in the form of discussion, explanation and reassurance. Some patients may require more intensive psychotherapy, either individually or in groups, depending on the nature of the psychological factors involved. Symptomatic measures include the relief of anxiety and agitation by sedatives such as meprobamate and amylobarbitone and the treatment of insomnia by the judicious use of hypnotics.

If the depressive illness is of such severity that the patient is prevented from carrying on with his work and normal activities, admission to hospital for treatment is usually desirable. Psychoanalytic therapy is ineffective and probably contra-indicated for severe endogenous depressive states for which the treatment of choice for many years has been convulsant therapy. Meduna[71] first introduced convulsant therapy, using chemical methods, which were later superseded by the electrical method of Cerletti and Bini[72]. The electrical method of producing therapeutic convulsions (electroplexy) is usually given once or twice weekly until the optimum therapeutic effects are achieved. The exact number of treatments varies with the individual and has to be judged according to the response and the needs of each case. Nowadays, electroplexy is given with general intravenous anæsthesia and short-acting muscle relaxants, which reduce the force and extent of muscular movements, thus eliminating risk of bony injury.

Another treatment sometimes used for depressive states is continuous narcosis, in which narcotics and sedatives are given to induce sleep for up to 18 hr. or so a day. This method sometimes shortens a depressive illness. Patients with depressive states developing after prolonged stress and associated with marked loss of weight often benefit from modified insulin therapy. This treatment consists of giving intramuscularly, gradually increasing doses of insulin to induce hypoglycæmia. In contrast to the insulin coma treatment for schizophrenia, the hypoglycæmia is terminated by glucose administration before it deepens sufficiently to cause loss of consciousness. When all other methods of treatment have failed, prefrontal leucotomy or one of its modern modifications may have to be considered for patients with long-standing depression associated with marked emotional tension. The best results are obtained in patients with good previous personality and whose social and home circumstances are favourable for resettlement and rehabilitation after the operation.

New Pharmacotherapeutic Agents

The available evidence on the actions and effects of the new anti-depressant drugs suggests that none is as effective as electroplexy for severe endogenous depressions. The amphetamines, pipradol and methylphenidate, are of very limited value and have a possible place only in the management of mild depressive states, but have serious disadvantages and unpleasant side-effects and are of no value for severe depressive states. With regard to the more recently introduced anti-depressant drugs, controlled trials have demonstrated that deanol is of no therapeutic value. Iproniazid helps some depressive states but its dangers do not justify its continued use. Of the new monoamine oxidase-inhibiting drugs, phenelzine is safer and quicker in action than iproniazid and is effective in some depressive states, mainly, in my experience, in depressive states of mild or moderate severity; it was not as effective as electroplexy for severe endogenous depressive states. Similarly, imipramine helps some depressive states, but again in my experience it is not as effective as electroplexy for severe endogenous depressions.

In my opinion it is not possible to select beforehand on the basis of clinical criteria which patients are likely to benefit from any of the new drugs. There is, indeed, an urgent need for further research to discover the relevant attributes of the individual, whether these prove to be physiological, chemical, psychological or constitutional in nature, to enable a more reliable selection of patients for specific pharmacotherapeutic agents.

In conclusion it may be said that the results of controlled studies on some of the new anti-depressant drugs, although not confirming the early enthusiastic claims of their advocates, are sufficiently encouraging to stimulate further research aimed at discovering safe drugs of greater potency and efficacy for the treatment of depressive states. The importance of such a discovery would indeed be great for the individual sufferer and for society generally.

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OBITUARIES

Mr. Harold Tongue, C.B.E.

Harold Tongue died in St. Thomas's Hospital, London, on February 18, after a long illness.

Educated at the Municipal Secondary School, Manchester, and the Manchester College of Technology, Tongue served his engineering apprenticeship jointly with Crossley Brothers, Ltd., and Kendall and Gent, Ltd. After Army service throughout the whole of the First World War, he joined the Co-operative Wholesale Society, Ltd., and eventually became chief engineer of the Research Department. In the early days of his career he followed the advice which, in later years, he gave frequently to young engineers, and gained experience by changing his job as opportunities arose. During this period he served with the British Portland Cement Research Association, the National Boiler and General Insurance Company, Ltd., the Chemical Engineering Department of the Chemical Research Laboratory (Department of Scientific and Industrial Research) and the London County Council.

At the beginning of 1945 he was seconded to the Department of Scientific and Industrial Research for work in Canada in the atomic energy field. First at Montreal, and later at Chalk River, Ontario, he took over the major engineering responsibility for the first high-power heavy-water moderated reactor to be built in that country. The subsequent great success of this venture owed much to his careful supervision.

While in Canada he joined in the early planning of the Atomic Energy Research Establishment, and in 1947 he went to Harwell as chief engineer to help realize what was to become one of the world's largest research establishments. His programme was large and diverse: in addition to establishing the equivalent of a small town on the Berkshire Downs, two research reactors, *Gleep* and *Bepo*, had to be designed, manufactured and built, and the Engineering Division had to provide strong supporting services to the scientific divisions. On the development side, special workshops were set up to solve the then novel problems of graphite machining, and for the casting of uranium rods, and their subsequent fabrication into fuel elements.

With the architects and engineers of the Ministry of Works he planned, for the first time in Great Britain, the novel services required to handle radioactive materials on a large scale. The best tribute to his vision is that these services, though extended, have not changed materially in basic design.

In those days a major problem was the handling of radioactive wastes, including waste disposal, decontamination, laundry services and transport of radioactive liquids, and a special branch of the Engineering Division was set up to handle these matters. Harold Tongue had the greatest regard for safety, and he personally led the important negotiations with the Thames Conservancy Board, the Metropolitan Water Board and the Ministry of Health, which resulted in agreement on the administrative and technical controls required for the safe disposal of radioactive effluent.

The Engineering Division included a large proportion of industrial employees, and it was natural that the chief engineer became responsible for the overall management of industrial labour, and chairman of the Joint Factory Committee. It was here that Tongue's strength of character and sense of justice brought respect from all concerned, and his philosophy on industrial management and human relations will live through his close associates, whom he influenced profoundly.

In 1950, after the Windscale plutonium-producing piles had been completed, there was a difficult choice whether to deploy available effort at Harwell on graphite-moderated gas-cooled reactors, or on the design and development of a fast reactor, for power production. Harold Tongue, giving great weight to safety considerations, and to the concept of steady development, believed that the gas-cooled system was the natural successor to *Bepo* and the Windscale

piles. The feasibility study of a gas-cooled system undertaken in 1951 and 1952 by his engineers, under Goodlet and Moore, in collaboration with Messrs. Babcock and Wilcox, Ltd., and Parolle and Wessoe, Ltd., laid the foundation for the present nuclear power programme in Britain.

In 1954 Tongue gallantly accepted medical advice to retire prematurely, although this was a severe blow to himself and his associates. Before leaving, however, he was able to see mature the first craftsmen and engineers from the apprenticeship scheme he founded. For his many contributions to atomic energy he was made C.B.E. in 1953.

Upon retirement he remained an active consultant to the Atomic Energy Research Establishment at Harwell. Happily he lived to see the publication of the second edition of his first book, "Design and Construction of High Pressure Chemical Plant". He was also the author of "A Practical Manual of Chemical Engineering".

P. BOWLES
R. H. BURNS

Dr. D. T. Germain-Jones

DR. DONALD THOMAS GERMAIN-JONES retired from the post of chief geophysicist of the British Petroleum Company in August 1959 at the early age of fifty-two. He was having a well-earned rest before taking another assignment when he met his death by drowning after a boating accident on the Thames on January 20. The news of the death of so young and active a man came as a great shock to his friends and colleagues in the geophysical world.

Dr. Germain-Jones was born on November 24, 1906, and was educated at the University of London. He was a Royal Scholar of the Royal College of Science and obtained a first-class associateship and a first-class B.Sc. degree in 1926. In the following year he was awarded the diploma of the Imperial College, and in 1937 he obtained a Ph.D. degree for his work on Iranian geophysical problems. He was a Fellow of the Institute of Physics, of the Royal Astronomical Society and of the Institute of Petroleum. He was an early member of the Society of Exploration Geophysicists, and a founder, and later president, of the European Association of Exploration Geophysicists. He contributed greatly to the successful launching of the journal, *Geophysical Prospecting*, of the latter Association, and wrote a number of articles for the *Journal of the Institute of Petroleum* on developments in exploration geophysics.

'D.T.' began his long and distinguished career in geophysics when he joined BP (Anglo-Persian Oil Co. as it then was) in Iran in 1927. Those were early days in seismic exploration, and field-work consisted largely of in-line refraction surveys with a Mintrop seismograph. In the 1930's, new instruments and techniques for arc shooting were evolved, which proved ideal for outlining rapidly the axes of the steeply folded structures such as Haft Kel, White Oil Springs and Gach Saran. In 1936, Dr. Germain-Jones discovered by these methods the Lali oilfield under a pronounced surface syncline; this achievement helped to establish the reputation within the Anglo-Persian Oil Co. of geophysics as a method of finding oil.

Dr. Germain-Jones was a leading figure in refraction work throughout this period. He established the fact that the producing Asmari limestone is a very poor carrier of refracted energy because of its limited thickness and that the refractor recorded in Iran was a much deeper Cretaceous (Rudist) limestone. He established methods of refraction interpretation by means of matching reversed in-line refraction shooting long before the publication of any papers on this subject.

His flair for organization and research led to the highly successful development of British Petroleum's first geophysical research station at Kirklington Hall, near Nottingham, and was later to aid him in the build-up of the Geophysical Division when he became chief geophysicist in 1953, at a time of great expansion of activities.

'D.T.' liked an outdoor life and sports. As a young man he was interested in swimming, walking and tennis, and when senior research geophysicist at Kirklington, he founded sports clubs for such pastimes as archery and rifle-shooting.

The untimely death of such an enthusiast will be a great loss to the oil exploration industry. Dr. Germain-Jones was truly a great pioneer.

He is survived by his wife and one daughter.

NEWS and VIEWS

Pharmacology in Leeds: Prof. D. R. Woods

DR. D. R. WOOD, who has been appointed to the chair of pharmacology in the University of Leeds in succession to Prof. W. A. Bain (see *Nature*, 182, 1550; 1958), is a graduate of Oxford in medicine and science and had worked in Prof. J. H. Burn's department there. He went to Sheffield as lecturer in pharmacology in 1948 and was promoted senior lecturer in 1952. He has worked for short periods with Dr. B. N. Halpern in Paris and with Prof. Georg Kahlson in Lund. During the academic year 1955–56 he worked in the Department of Pharmacology at Yale University School of Medicine. In September 1957 he took up his present post as associate professor of pharmacology at McGill University. Dr. Wood's main interests have been in gastric secretion and in antihistamine drugs, but he has collaborated extensively with other workers particularly in the biochemical field. In addition to his research and teaching duties, he served as secretary of the British Pharmacological Society during 1952–57.

Education at Sheffield: Prof. Boris Ford

MR. BORIS FORD has been appointed director of the Institute of Education and professor of education in the Institute, University of Sheffield. Mr. Ford was educated at Gresham School, Holt, and the University of Cambridge, where he graduated with first-class honours in the English Tripos in 1939. Mr. Ford served in the Army during 1939–46, and from 1946 until 1951 was successively chief editor, deputy director and finally director of the Bureau of Current Affairs. In 1951 he was appointed information officer of the Technical Assistance Board of the United Nations, and during 1953–55 he was secretary of the national inquiry into liberalizing technical

education. From 1955 until 1957 he was part-time secretary of Children's Play Activities and editor of the *Journal of Education*. In 1957 he was appointed first head of school broadcasting, Independent Television. Mr. Ford left this post in 1958 to take up his present appointment as education secretary, Cambridge University Press. Mr. Ford has written a number of books, and since 1949 he has been general editor of the Pelican "Guide to English Literature" and editor of *The Universities Quarterly* since 1955.

Patents, Designs and Trade Marks (India): Dr. S. Venkateswaran

Dr. S. Venkateswaran has been appointed first controller-general of patents, designs and trade-marks (India). He graduated in the University of Madras in 1919, and during 1919–21 was Government of Madras Research Scholar at the Indian Institute of Science, Bangalore. In 1922 he was appointed research assistant in the Agricultural Research Institute, Pusa, and during 1923–30 he worked as chemical assistant at the Government Test House, Alipore. During 1924–31 he carried out scientific research under Sir C. V. Raman and published several research papers in the *Proceedings of the Royal Society*, *Philosophical Magazine*, *Nature* and other well-known scientific journals. He took the D.Sc. and B.L. degrees and was elected a Fellow of the Indian Academy of Sciences. In 1930 Dr. Venkateswaran was appointed examiner of patents at the Patent Office, Calcutta. During this period he published a valuable treatise on the "Trade and Merchandise Marks Law in India" in 1935. This book was largely responsible for the introduction of the first trade marks legislation in India in 1940, and the Trade Marks Bill of 1940 was based on the recommendations made by him. Another book, on "Cotton Trade Marks", was published by him in 1939. In September 1940 he was appointed assistant registrar of trade marks, and, in 1944, deputy registrar of trade marks. During this period he published a treatise on the "Trade Marks Act, 1940", and a supplement in 1953. In 1954 he was appointed registrar of trade marks. In February 1957 he became officer on special duty (trade marks, patents and designs). On February 8, 1959, he took charge of both the Trade Marks Registry as registrar of trade marks and of the Patent Office as controller of patents and designs.

Captain Robert Dexter Conrad Award: Dr. R. E. Gibson

The fourth Captain Robert Dexter Conrad Award, consisting of a gold medal and citation, was presented to Dr. R. E. Gibson, director of the Applied Physics Laboratory, Johns Hopkins University, by the Assistant Secretary of the Navy at the Navy Science Symposium in Pasadena, California, on March 9, 1960. The Conrad Award is made annually in recognition of outstanding scientific contributions to the United States Navy in memory of Captain Conrad, one of the principal architects of the programme in basic science conducted by the Office of Naval Research, U.S. Navy, for more than a decade. Dr. Gibson was born in King's Lynn, Norfolk, England. He studied chemistry under Sir James Walker in the University of Edinburgh, holding a Carnegie Research Fellowship, and was awarded a Ph.D. In 1924 he joined the staff of the Geophysical Laboratory of the Carnegie Institution of Washington. There he served as a physical chemist until 1946, except for a five-year period, 1941–46, when he was on leave of absence to engage in war-time research and development, being, among other things, the first director of research, Allegany Ballistics Laboratory, Cumberland, Maryland. In January 1946 he went to the Applied Physics Laboratory, Johns Hopkins University, became acting director in July 1947, and director in April 1948. He has published articles on physical chemistry, high-pressure chemistry, rockets and administration of research and development.

Marine Nuclear Propulsion for the United Kingdom

In replying for the Government in an adjournment debate in the House of Commons on March 21 on marine nuclear propulsion, the Joint Parliamentary Secretary to the Ministry of Transport, Mr. John Hay, said that in 1957 the Government set up a Committee to go into the question of the use of nuclear power in ships and to make recommendations. The chairman was Mr. T. E. D. Galbraith, the Civil Lord of the Admiralty, and shipowners, shipbuilders, the Atomic Energy Authority, Lloyd's Register and Government Departments concerned were represented on the Committee. Mr. Wood said that the Minister of Transport had decided to keep the Committee in being, that Lord Chesham was now its chairman, that the Committee would be served by a Technical Committee set up last year with the Director-General (Ships) at the Admiralty as chairman. With full approval of the Government the Galbraith Committee decided deliberately to concentrate upon the ultimate objective of building a nuclear ship which would be economic to operate. Further, at present, the lowest power worth considering was 20,000 shaft horsepower, which was sufficient to propel a tanker of 65,000 deadweight tons. Most ships to-day and in the foreseeable future were much smaller and a second objective was thus the development of economic nuclear-propelled units of progressively smaller power. In May 1959 the Committee received eight papers and models produced by the Atomic Energy Authority and industrial consortia relating to six different reactor systems. The Technical Sub-committee evaluated the merits of the three systems which appeared to be the most developed in timing and technology: the pressurized water reactor, the boiling water reactor, and the organic liquid moderated reactor. The Technical Sub-committee regarded the boiling-water and the organic liquid moderated reactor systems as the most promising at the time and recommended that competitive tenders should be invited from selected firms for both systems. Invitations to tender for a reactor to power a tanker of 65,000 tons deadweight were issued to five firms in February against a closing date for July 29, 1960, and in the meantime detailed investigations were being made on safety measures for such ships.

Estimated U.K. Power Output 1960–65

In a reply circulated in *Hansard* for March 21 the Minister of Power, Mr. R. Wood, gave the total output capacity of generating plant of the Central Electricity Board expected to be in operation at the end of the next five years as 27,130 MW. (1960); 29,359 MW.; 31,143 MW.; 32,799 MW.; and 34,833 MW.; the nuclear proportion would rise from 2·0 per cent (1961); 2·6 per cent (1962); 4·0 per cent (1963); to 5·2 per cent, and the estimated proportion of total power stations output to be provided by nuclear

plant in 1964–65 was 8 per cent. New nuclear generating plant coming into operation was expected to be 575 MW. (1961); 250 MW. (1962); 500 MW. (1963) and 500 MW. (1964), bringing the total new generating capacity for these years to 2,553; 2,096; 2,069; and 2,377 MW., respectively.

Training Services in Industry

THE Industrial Training Council has issued a booklet, "Training Services in Industry", describing first the training services provided by employers' organizations and joint bodies and then some examples of the services available in particular industries (pp. 39. London: Industrial Training Council, 1959. 1*s.* 6*d.*). There is also a section dealing with the services provided by other bodies, such as the Ministry of Labour, the City and Guilds of London Institute, the British Association for Commercial and Industrial Organization, the Industrial Welfare Society, the Institute of Personnel Management and the National Institute of Industrial Psychology. In a brief introduction, the chairman of the Council, Lord McCorquodale of Newton, points out that the Council is devoting attention in the first instance to the problem presented by the coming bulge in school-leavers, the solution of which will depend largely on the action taken by individual employers, and demands accordingly a clear lead from the employers' organizations working in close co-operation with the trades unions.

Research at the Imperial College of Science and Technology

THE fifty-second annual report of the Governing Body of the Imperial College of Science and Technology, covering the year ended July 31, 1959, includes this year for the first time a detailed report from the rector, Sir Patrick Linstead, which contains some notes on the main trends of the research work carried out during the year, towards which some £320,000 was received in grants for research and from research contracts, of which £110,000 was from the Department of Scientific and Industrial Research (Fifty-second Annual Report of the Governing Body, 1958–59. Pp. ii+102. London: Imperial College of Science and Technology, 1959). A more detailed account of the work in progress is available in the third of the triennial research reports covering the period 1956–59 (Imperial College of Science and Technology (University of London). Research Report of the Royal College of Science, 1956–1959. Pp. v+77. London: Royal College of Science, 1959). Commenting on the expansion of the College, it is emphasized that while with 1,638 undergraduates and 903 postgraduate students, the College is a year ahead of the expansion planned for 1957–62, the available accommodation per student has declined steadily and sharply over the past six years to a level only three-fifths of that in the immediately preceding years and to less than half of that before the War, in consequence of the long time required to provide additional accommodation, and this has involved additional strain on the academic staff, whose numbers have not risen at the rate envisaged in the expansion plan. Of the sixteen additional members in the session, three are professors and six readers, bringing the number of professors to thirty-nine and of readers to sixty-eight, and seven of these nine appointments were in departments of engineering. The policy of broadening the studies of undergraduate students continued and there are now forty-three full-time postgraduate courses. Appendixes to the report include the Commemoration Day address of the Special Visitor, Lord Bridges, and lists of publications, 1958–59, and of lectures given by visiting lecturers and, outside the College, by members of the staff.

Higher National Certificates and Professional Status

IN the twelfth annual report of the Southern Regional Council for Further Education attention is directed to the changing role of higher national certificates in connexion with professional qualifications (October 1959. Pp. 40. Reading: Southern Regional Council for Further Education, 1959). Whereas the roads to professional qualifications in technology previously included higher national certificates as well as university degrees and professional examinations, there is now a set of circumstances in which the way through part-time study is becoming longer, more arduous and more hedged about with changing conditions. The internal university degree, the higher national diploma and the diploma in technology lead readily enough, subject to the views of professional institutions, to professional status, but these require attendance at full-time or sandwich courses. In the past the great majority of professional engineers trained via the route of part-time studies and, although provision of more and more sandwich courses is a desirable development, it would be unfortunate if the part-time route were to be made impossibly difficult. The question must be asked: Has the stage now been reached when potential professional technologists of real ability are being prevented from reaching their proper goal?

The Cawthron Institute

THE annual report 1958–59 of the Cawthron Institute, Nelson, New Zealand, besides the Director's report and the financial balance sheet, contains nine special articles dealing with chemical investigations on bitter pit of apples, the nutrition of *Phytophthora cactorum* (L. and C.) Schroet, water-culture experiments on nutritional problems of the tomato, treatment of tomato seedlings with *n–m* tolylphthalamic acid, influence of tomato seedling treatment on flowering and fruiting of lower trusses, pasture investigation on Pakihi land at Onekaka, pasture trials at eighty-eight valley, aspects of the Cawthron Institute Museum and the taking of soil samples for advisory purposes (pp. 46. Nelson: Cawthron Institute, 1959). The Director's report refers to an extensive series of analyses of soils from alluvial areas of the Waimea Plain in progress and the continuance of trials with nitrogenous substances for hops. Chemical investigations on tobacco were concerned chiefly with the effect of fertilizer treatment and variety on the composition of curved leaf of the 1957–58 tobacco crop. Tomato research included further trials of chloropicrin treatment, the cost of which is now only half that of steam sterilization, and of sprays of magnesium, boron, iron, manganese, copper, zinc and molybdenum, none of which showed material and consistent benefit on foliage or crop. Application of large volumes of water and small amounts of fertilizer appear to be the most important factors influencing the development of cloud in tomatoes, and shading appeared to be detrimental when natural light was meagre but beneficial during prolonged periods of bright sunshine; higher nitrogen tended to improve the quality of late-harvested fruit. A list of publications is included.

Report of the Smithsonian Institution

The annual report of the Board of Regents of the Smithsonian Institution for the year ended June 30, 1958, contains besides the Secretary's report and those of the several branches, the general appendix, comprising a miscellaneous selection of papers, some of which are original (pp. x+559+107 plates. Publication 4354. Washington, D.C.: Government Printing Office, 1959. 3.75 dollars). Among those not available elsewhere or not easily accessible to British readers may be mentioned those of Farrington Daniels on "The Sun's Energy"; D. Cox and M. Stoiko, "Rocketry"; D. S. Jenkins, "Fresh Water for Arid Lands"; Prof. H. E. Suess, "The Abundance of the Chemical Elements"; Prof. K. E. Bullen, "Earthquakes and Related Sources of Evidence on the Earth's Internal Structure"; P. B. Sears's Condon Lecture, "The Ecology of Man"; E. F. Knipling, "Screw-worm Eradication: Concepts and Research leading to the Sterile-Male Method"; W. L. Schmitt, "Narrative of the 1958 Smithsonian–Bredin Caribbean Expedition"; A. I. Hallowell, "The Backwash of the Frontier: the Impact of the Indian on American Culture"; Prof. R. E. Crist, "Acculturation in the Guajira"; N. M. Judd, "The Braced-up Cliff at Pueblo Bonito"; H. G. McCurdy, "The Childhood Pattern of Genius"; and A. N. B. Garvan, "The New England Porringer: an Index of Custom".

The National Research Council (Canada)

The "Review of the National Research Council" (Canada), 1959, describes the work of the Council during the year ended December 31, 1958, but includes some later work (pp. 337. NRC No. 5251. Ottawa: Queen's Printer, 1959. 75 cents). It contains reports of the directors of divisions and heads of sections, as well as accounts of the work of the Council's committees and a list of scientific staff. Supplementing the previously published annual report of the Council, it is distributed to scientists, research institutions and other persons or organizations who require more detailed information.

Journal of Electroanalytical Chemistry

The appearance of a new journal cannot nowadays be greeted with unqualified enthusiasm. However, the splintering of the sciences into highly specialized parts seems to be inevitable, and literature research within each speciality would certainly be simplified if the workers concerned could be persuaded to publish their contributions in the particular journal devoted to their subject. A new *Journal of Chromatography* appeared in 1958, and its success has encouraged the publishers (Elsevier, Amsterdam) to bring out an accompanying *Journal of Electroanalytical Chemistry*. Both these fields are of great importance in modern analytical chemistry, and hitherto research in both of them has been spread throughout the chemical literature. The format of the new *Journal* is convenient, the editors (Profs. G. Charlot, J. O'M. Bockris and C. N. Reilley) are distinguished, the editorial board contains most of the internationally known electrochemists, and the contents of the first issue set a high standard. Quite generally, the publishers are to be congratulated on the new venture. Nevertheless, the increasing flow of papers poses many problems; the growing sentiment is that the necessity is not for new journals but for a more critical approach in the editing and refereeing of papers in existing journals. The essence of too many papers in the general literature could be conveyed in two lines rather than two pages. Only time will show whether or not this new *Journal* will fulfil its purpose. If the bulk of electroanalytical papers can be diverted into it and if the editors make sure that the initial high standard is maintained, then the *Journal* will indeed be a worthy contribution to the literature.

Biochemical and Biophysical Research Communications

This new journal aims at securing rapid publication of short but well-documented communications in all fields of experimental biology (Vol. 1, No. 1 (July 1959). Pp. 44. Published monthly. Subscription rates (1 volume, 6 issues): Institutional subscribers 12 dollars; 85*s.* 6*d.* Private subscribers certifying that the volume will be for personal use only, 6.50 dollars; 46*s.* (New York and London: Academic Press, Inc., 1959.)) The scope of the communications so far published is not unlike that of many of the biochemical "Letters" to *Nature*. The papers are reproduced directly from the typescript, and in spite of differences in size of type, the appearance of the pages is pleasing and the quality of reproduction of typescript and figures is excellent. So far, the number of communications per monthly part has ranged from about ten to twenty, each of about four double-spaced typescript pages. The aim of quick publication is certainly being achieved: for example, in the January 1960 number, the earliest paper was received on November 23, 1959, and the latest, on January 7, 1960.

Cost of Scientific Periodical Publications

Mr. K. W. Humphreys, honorary secretary of the Standing Conference of National and University Libraries, and Mr. H. D. Barry, secretary of the Library Association, have written referring to the high cost of scientific periodicals. They have prepared the following table of periodicals which are comparable in that none has many advertisements and they are similar in matter per page; they have arranged them in order of the price per page:

Title	Source	Publisher	Price per page
Biochemical Journal	U.K.	Camb. Univ. Press	0·75*d.*
Journal of the Chemical Society	U.K.	Chemical Society	1·0
Chemical Reviews	U.S.A.	American Chemical Society	1·2
Journal of Soil Science	U.K.	Oxford Univ. Press	1·3
Information and Control	U.S.A.	Academic Press	2·0
Philosophical Magazine	U.K.	Taylor and Francis	2·2
Biochemica	Dutch		2·25
Fuel	U.K.	Butterworth	2·5
Journal of Electronics	U.K.	Taylor and Francis	2·8
Chemical Abstracts	U.S.A.	American Chemical Society	3·0
Journal of Inorganic and Nuclear Chemistry	U.K.	Pergamon Press	3·8
Tetrahedron	U.K.	Pergamon Press	4·0
Physics and Chemistry of Solids	U.K.	Pergamon Press	4·1
Chemical Engineering Science	U.K.	Pergamon Press	5·8
Reactor Science	U.K.	Pergamon Press	8·9

They believe that a reduction in costs leads to increased circulation of scientific journals.

Preservation of Documents

A study by W. K. Wilson and B. W. Forshee, of the National Bureau of Standards, made at the request of the National Archives, the United States Army Map Service, the Library of Congress and the Virginia State Library, of the preservation of documents by lamination has shown that pre-treatment with alkaline media is desirable if the paper contains an appreciable amount of acid (Monograph No. 5.

Pp. ii+20. Washington, D.C.: Government Printing Office, 1959. 20 cents). The lamination process does not degrade cellulose acetate film to a measurable extent. Acid-free papers are not degraded during lamination, but papers containing acid are degraded in proportion to the amount of acid present. Addition of tissue to the laminate increases the tensile strength and internal tear resistance, but decreases edge-tear resistance, compared with that of film alone projecting beyond the paper. Loss of plasticizer with time from a cellulose acetate laminating film does not impair the properties of the laminate. A specification is included for a cellulose acetate laminating film for the purpose, and preliminary results with polyethylene and polyethylene terephthalate are described.

Education and Training in Chemical Engineering

As a supplement to its booklet, "Chemical Engineering: a Career", the Institution of Chemical Engineers has now issued a supplement entitled "Education and Training in Chemical Engineering", which brings together information published from time to time in its leaflets (pp. 47. Supplement to "Chemical Engineering: a Career". London: Institution of Chemical Engineers, 1959). The information covers scholarships in chemical engineering; lists of university and college scholarships and of industrial organizations with scholarship schemes in chemical engineering; courses in chemical engineering in the United Kingdom; notes on practical training of student apprentices and on graduate training in chemical engineering in the United Kingdom; and lists of training schemes for student apprentices, and also for graduates, in chemical engineering in the United Kingdom. Copies are available free of charge on written application to the Institution.

Vivisection

THE Research Defence Society has published a pamphlet giving its views on common misapprehensions about vivisection. This is done by posing a series of questions and providing answers which approximate as closely to the truth as biological research has so far revealed. Among the questions are: What is vivisection?; How is it possible to avoid causing pain to animals?; How does one know that an animal is not suffering pain?; What experiments are performed without anæsthetics?; How many experiments are performed each year on animals?; What sort of results come from experiments on animals and what advances have been brought about by animal experiments? The Society is to be congratulated for the reasoned nature of its reply to the particular question: Why do anti-vivisectionists oppose animal experiments? The pamphlet can be obtained from the Research Defence Society, 11 Chandos Street, Cavendish Square, London, W.1, price 6*d*.

Effects of Radiation on Human Heredity

THE first meeting of the World Health Organization Expert Committee on Radiation was held in Geneva during July 1958. It put forward the suggestion that a study of populations normally exposed to large amounts of background radiation would be useful as a source of information on the genetical effects of radiation on man. At the same time such a study should also provide information on somatic radiation damage. From a survey of known areas of high background radiation, the Kerala area on the south-west coast of India was chosen as the only suitable one. Along a hundred miles of coastline in this area the sands contain radioactive monazite, the main source of radioactivity being thorium and its decay products. The population, estimated at 80,000, receives 10–15 times as much background radiation as the average person elsewhere. The Committee laid down general principles for the planning of the 'Kerala project'. It recognized that a comparison between the frequencies of specific mutant phenotypes in the irradiated and control populations will scarcely be feasible. Instead, it will be necessary to rely on comparisons of populations with respect to features like sex-ratio, life-span, fertility, etc., which are assumed to be influenced by mutations in numerous genes. A plan is drawn up for the levels on which the investigation should be conducted. The general statistical aspects of the problem are considered, and a rough estimate is made of the requirements in money and personnel.

Home Range in a Feral Goat Herd

THANE RINEY and Graeme Caughley, of the New Zealand Forest Service, Wellington, New Zealand, have studied the movements of a small population of free-ranging feral goats (*Capra hircus*) for sixteen months, advantage being taken of individual natural markings adequate for identification of each animal (*New Zealand J. Sci.*, **2**, No. 2; June 1959). In the winter, adult females went into the forest, leaving the nanny–kid herd in the more open area, where study was concentrated. This was an area common to all nanny and kid home ranges, and more than 90 per cent of all observations were made in this herd-range, which was 1,050 yards at its widest diameter. The herd-range consisted of three high-use areas surrounded by areas of less use. In the nanny–kid herd, two groupings were recognized—the family group, and the larger more temporary associations formed within the herd. The herd was never observed all together at any one time. There was no antagonism between groups or associations, and although some aggressive behaviour occurred among billies, no signs of leadership emerged. The boundary of a herd-range appears to be more in the nature of a band or zone than a line, and in this area it is apparently influenced by the availability of cover. Relations between sheep and goats do not appear to affect boundary formation and shooting has not been intensive enough permanently to alter the shape of the herd-range. The nanny–kid herd is considered the part of the population most suitable for the application of management and control measures.

Biology Staining Schedules

R. R. FOWELL's well-known booklet on the practical details of staining techniques designed for students of intermediate degree standard has been issued in the sixth edition (H. K. Lewis and Co., Ltd. 3*s*. net). Because of the increased importance given to microbiology in medicine, industry and other fields, facilities for training in the subject are now provided by a number of technical colleges and universities. In view of these developments, an additional chapter, entitled "Microbiology Schedules", has been incorporated in the present edition, and the appendix has been extended to include formulæ for the stains mentioned in these schedules. The staining methods advocated have been limited to those considered most useful for routine purposes. Additional information on dehydration has also been included.

Agricultural Research Council : Studentships and Fellowships

A BOOKLET issued by the Agricultural Research Council gives details of the postgraduate studentships and research fellowships offered by the Council in 1960 (Pp. 16. London : Agricultural Research Council, 1960). Besides 30 studentships tenable for one to three years, three junior agricultural research fellowships tenable for three years, valued at £700–£1,000 per annum, are offered and two veterinary research fellowships tenable for three years, rising from £900 to £1,000. The studentships, which cover training in agricultural engineering, dairy engineering and statistics, and a limited number of veterinary training grants for four years duration to enable science graduates to read for a veterinary qualification, are for £340–£520 per annum, tax free, together with educational fees. The booklet also outlines the scope of agricultural research and the openings available in agricultural research and advisory work. Of the 407 awards since 1932, 43 are still current ; 153 holders have entered on careers in agricultural research or advisory services at research institutes, etc., 88 have taken up similar university appointments and 45 overseas appointments.

Research Corporation of the United States

THE Research Corporation was founded by Frederick Gardner Cottrell, scientist, teacher and inventor, in 1912. Cottrell gave the proceeds from his inventions in the electrostatic precipitation of gas particles as an endowment to make grants for scientific research. Since 1912 more than 2,500 scientists have been aided by grants to institutions in the United States and in other countries totalling more than 13 million dollars. During 1959 the Foundation distributed approximately 1·2 million dollars for 383 grants. It is announced that grants ranging from 1,100 dollars to 12,000 dollars have already been awarded to forty-three United States and foreign colleges for the current year. So far forty-eight grants have been awarded for research : in chemistry (34), physics (9), astronomy (2), and the biological sciences (3).

The Swinburne Award

THE Council of the Plastics Institute announces that the Swinburne Award, instituted in 1959 and consisting of a gold medal, a certificate and a sum of money, will now be an international award. The award will be made to persons, whether members or non-members of the Institute, who shall have made an outstanding contribution to the science or technology of plastics or of plastics engineering.

Asia Foundation Grants

THE American Geographical Society announces that it has received a grant of 2,500 dollars from the Asia Foundation to aid Asian libraries and Asian scholars temporarily in the United States. The grant will be used to provide selected libraries with the society's journal, *The Geographical Review*. In addition, small stipends, ranging up to 300 dollars, will be offered to students and scholars currently in the United States, to enable them to do research at the society. Applicants must be at least at the graduate level and may come from any Asian country from Afghanistan eastward, except for Australia, New Zealand, and territories of the South Pacific. Further information can be obtained from Dr. Charles B. Hitchcock, director of the American Geographical Society, 156th Street and Broadway, New York City.

Competition for Science Teachers

THE journal *Teaching Science* has organized a competition for science teachers. Entries are invited for articles concerning a new technique or content in classroom work, the development of special interest in the pupil, the fostering of the outstanding child or the development of the backward child, or the encouragement of group activities in the classroom or outside. The fostering of special relations with industry or the local museum or library, or the use of new materials, is included. £100 in prizes are offered to cover two groups of entries, namely, an 'S' group which includes all types of Secondary Schools up to O-level G.C.E., and 'P', Primary Schools. In each group there will be four awards totalling £50. The awards will be made by an independent committee. The closing date for entries is May 31. Further information can be obtained from T.S.T.S., *Teaching Science*, 5 Great James Street, London, W.C.1.

Australian Irradiation Conference

THE Australian Atomic Energy Commission is holding a conference on the Technological Use of Radiation at the University of New South Wales during May 23–25. The object of the conference is to review recent developments and to stimulate research and interest in the technological applications of radiation. It is intended that the conference should cover the following topics : food preservation ; sterilization of medical supplies ; sterilization of industrial raw materials ; uses in the chemical industry (polymerization, modification of polymers, promotion of chemical reactions, etc.) ; insect pest control ; genetic applications. Further information can be obtained from the Secretary of the Irradiation Conference, Australian Atomic Energy Commission Research Establishment, Private Mail Bag, Sutherland, New South Wales, Australia.

University News : Leeds

THE annual report of the Librarian, University of Leeds, for the session 1958–59, records accessions during the year of 20,213 volumes, 2,623 pamphlets and 29,721 periodical parts, bringing the total holdings to 639,249 books, of which 439,987 are in the Brotherton Library, 35,879 in the Medical Library and 39,890 in the Agricultural Library (pp. 12. Leeds : The Brotherton Library, The University, 1959). Inter-library loans comprised 1,496 books borrowed and 3,163 lent. The third stage of the mezzanine floor construction, providing shelving for 70,000 volumes and 44 study rooms, was completed for the beginning of the 1958–59 session, and the seating capacity of the Brotherton Library is now about 700 with some 275 further seats in sectional libraries. Other accommodation, however, is also required, such as a new co-ordination of catalogue, reference books and counter on a much larger scale and entirely outside the Reading Room. A fall of 4 per cent in cataloguing output is attributed to staff changes and to increasing claims on cataloguers' time.

Oxford

GRANTS are announced from the following bodies : *Department of Scientific and Industrial Research*,

£1,170 for the purchase of apparatus for the structural investigation and synthesis of peptides, to be carried out in the Dyson Perrins Laboratory under the direction of Dr. G. T. Young; £10,800 for the purchase of an electron-microscope, for use in research in the Department of Metallurgy under the direction of Prof. W. Hume-Rothery; £7,450 for work being carried out in the Clarendon Laboratory under the direction of Dr. N. Kurti; and £940 for work on the expansion of X-ray fluorescence analysis to lower atomic numbers being carried out in the Research Laboratory for Archæology and the History of Art; *National Coal Board*, £1,600 for the continuation of research on hot and humid conditions, being carried out in the Department of Human Anatomy under the direction of Dr. J. S. Weiner; *National Institute for Research in Nuclear Science*, £10,450 for an investigation of the alignment of nuclei for the proton linear accelerator, to be carried out in the Clarendon Laboratory under the direction of Prof. D. H. Wilkinson; *British Iron and Steel Research Association*, £1,000 for the continuation of research on the melting- and freezing-points of alloys, being carried out in the Department of Metallurgy under the direction of Prof. W. Hume-Rothery; and the *Mental Health Research Fund*, £3,330 for research on the physiology of drive, to be carried out in the Institute of Experimental Psychology by L. J. Herberg.

Announcements

Mr. I. E. Efford, of the Bureau of Animal Population, University of Oxford, has been appointed by the Royal Society to a John Murray Travelling Studentship in Oceanography and Limnology for 1960 to enable him to study the biology of a marine mite (Halacaridae) by means of field experiments at the Scripps Institution of Oceanography, La Jolla, California, U.S.A.

Mr. J. H. Gunlake has been elected president of the Institute of Actuaries in succession to Mr. F. M. Redington, whose term of office will expire on June 27. Other honorary officers who have been elected for the session 1960–61 are: *Vice-presidents*, N. C. Turner, F. H. Wales, F. H. Spratling, J. B. H. Pegler; *Treasurer*, A. W. Joseph; *Honorary Secretaries*, R. E. Beard, G. V. Bayley.

Miles Laboratories, Ltd., are establishing a basic research programme in England to supplement that already carried out by Miles Laboratories Inc. in the United States. The new laboratories will be at Stoke Court, Stoke Poges, Buckinghamshire, and they will provide facilities for fundamental work in chemistry, biochemistry and pharmacology, including necessary animal accommodation. The primary aim of the new Miles-Ames laboratories will be the development of diagnostic tests for the detection of diseases and the development of new therapeutic agents.

The annual conference of the Institute of Sewage Purification is to be held at Scarborough during June 21–24. Further information can be obtained from the Joint Secretaries, Institute of Sewage Purification, 10 Cromwell Place, London, S.W.7.

The summer meeting of the Institute of Mining Engineers is to be held in Cardiff during June 29–July 1. Further information can be obtained from the Secretary, Institute of Mining Engineers, 3 Grosvenor Crescent, London, S.W.1.

The Welsh Soils Discussion Group is arranging a field-meeting to be held on April 27, when visits will be paid to deciduous and coniferous forests in North Wales. Particulars can be obtained from J. A. Taylor, Department of Geography, University College, Aberystwyth.

A symposium will be held in Milan during June 3–4 for the purpose of reviewing the present status of biological and clinical research on Drugs affecting Lipid Metabolism. The symposium is sponsored by the Institute of Pharmacology of the University of Milan. Further information can be obtained from Prof. S. Garattini, Institute of Pharmacology, Via A. del Sarto 21, Milan.

The fifth Nuclear Congress, promoted by le Comitato Nazionale per le Richerche Nucleari and within the framework of the seventh International Electronics and Nuclear Exhibition, will be held in the E.U.R. Congress Palace, Rome, during June 20–June 26. Further information can be obtained from the General Secretary, Comitato Nazionale per le Richerche Nucleari, Rome.

A special Summer Laboratory Course in Techniques and Applications of the Electron Microscope is to be held for qualified senior biologists and medical research workers under the direction of Prof. Benjamin M. Siegel at Cornell University's electron microscopy laboratory during June 13–July 1. Further information can be obtained from Prof. Benjamin M. Siegel, Rockefeller Hall, Cornell University, Ithaca, New York.

A conference on the Mechanisms of Peroxide Reactions has been organized under the sponsorship of the Office of Ordnance Research of the U.S. Army and Brown University. It will be held during June 15–17 at the Metcalf Chemical Laboratories of Brown University in Providence, Rhode Island. Further information can be obtained from Prof. J. O. Edwards, Metcalf Research Laboratory, Brown University, Providence 12, R.I.

A conference on Coherence Properties of Electromagnetic Radiation is to be held at the Institute of Optics, University of Rochester, New York, during June 27–29. The conference is sponsored jointly by the United States Air Force, the Optical Society of America and the University of Rochester. Further information can be obtained from Miss H. Tobin, Institute of Optics, University of Rochester, Rochester 20.

The third symposium on Gas Chromatography, organized by the Society for Analytical Chemistry and the Gas Chromatography Discussion Group under the auspices of the Hydrocarbon Research Group of the Institute of Petroleum, will be held at the Assembly Rooms, Edinburgh, during June 8–10. Further information can be obtained from L. Brealey, Boots Pure Drug Co., Ltd., Standards Department, Station Street, Nottingham.

The French National School of Geographical Sciences (Ecole Nationale des Sciences Géographiques, part of the National Geographical Institute) will conduct a theoretical and technical course on aerial photogrammetry during June 20–29 at Saint-Mandé. The number attending is limited to 25. Applications for registration must be sent to the Director, l'Ecole Nationale des Sciences Géographiques, 2 Avenue Pasteur Saint-Mandé, before May 15.

THE NATURE CONSERVANCY

THE tenth annual report of the Nature Conservancy, covering the year ended September 30, 1959*, looks back over the Conservancy's first decade of effort undismayed by the major defeat experienced during the year in the loss of the unique cuspate foreland and migration cross-roads at Dungeness to the Central Electricity Generating Board for a nuclear power station. Although what has yet been achieved is regarded as no more than preparatory to the adoption in practice of conservation as a national policy in Britain, and no firm assurance for the future of the existing eighty National Nature Reserves, embracing more than 137,794 acres, can be found in the report, the Conservancy's target of a quarter of a million acres during the next few years is unchanged. Possibly the growing emphasis on the scientific character of the Conservancy's work which has been manifest in recent months, for example, in connexion with Lord Hailsham's appointment as Minister for Science, may ultimately have some effect; but meanwhile the chairman, Mr. A. B. Duncan, does well to point out that the task of mobilizing the resources needed for the exploration and study of the conditions and potentialities of life on land for plants and animals, including man, has been left very late.

The very fact that the task calls for high scientific standards, for trained research workers in many disciplines able to collaborate towards common scientific ends, quite apart from the necessary material resources, especially land in the form of carefully selected Nature reserves, suitable accommodation, equipment and money, gives the report a wide scientific interest; and scientific workers of varied interests may fairly be expected to share in the task of public exposition of the Conservancy's work and needs and the essential place in the national economy. It is encouraging to note that during the year the Conservancy was able to approve fifteen new grants to university scientists, totalling £25,084, and seven existing grants were renewed or extended. It is to be hoped that the Minister for Science will himself be mindful of the comments of the Select Committee on Estimates and see that adequate funds for such grants are in future available. Twelve awards of studentships were made, and there was a reserve list of six qualified candidates.

Scientific research covered a wide range. At Moor House, Westmorland, work continued on changes in floral composition of grassland communities after the exclusion of sheep grazing, and an outcrop of limestone has provided a wide range of habitats giving suitable environments for many of the scarcer montane plants. A further experiment was started to raise by management the biological status of an adjacent limestone soil to that of the Moor House meadow, and work continues on peat and peat vegetation, while in Snowdonia the general inferences drawn from earlier work on the intensity of sheep grazing in relation to environment have been substantiated, and in work on the effect of grazing on vegetation at Cwm Idwal and in the Snowdon area it is becoming increasingly apparent that grazing only in summer favours the spread of *Nardus* at the expense of more palatable species. The first part of the Scottish Vegetation Survey has been completed for publication, and in the recording of grassland transects of southern England on the whole the changes were like those previously reported—increases in more palatable grasses, in leguminous plants and in the seedlings of woody plants. Most of the work of the Plant–Soil Section at Merlewood on tree growth, forest productivity and soil type was devoted to completing outstanding short-term experiments. In an attempt to determine the best method of sampling a forest to determine dry matter and chemical content, an intensive study was made on a pine stand near Yedburgh, and two Forestry Commission thinning trials have been studied to determine the effects of past thinning practice on the weight of average-sized trees, ground flora and the accumulation of litter on the forest floor. Decomposition processes in the litter of the forest floor continue to form an important topic for research at Merlewood, and other work on the oak and ash woods of northern England includes a study on the Roudsea sites of the chemical changes occurring in ash litter enclosed in fine mesh nylon nets to minimize the effects of soil animals, the study of the fungal succession in decaying bracken rachides, and research on the defoliation of oaks by caterpillars of the oak-leaf roller moth (*Tortrix viridana*) in which the phosdrin injection technique yielded some unexpected results. At the Cairngorms nursery the advantage of low-voltage soil heating in lengthening the growing season is being investigated, using wind-generated current.

A programme of field and laboratory work to extend over the next few years has been commenced on Soay sheep by a team on St. Kilda, and a field study has been commenced of the ecology of British reptiles, particularly their habitats, hibernation areas, behaviour, predators and food, so that their conservation can be put on a more scientific basis. Initially the work, which is an integral part of the conservation programme in the management plans of the Hartland and Morden National Nature Reserves in Dorset, is being confined to three species of snakes and to the sand lizard (*Lacerta agilis*). Research has been started on the population dynamics of the red deer herds on the Isle of Rhum. The extensive programme of dune reclamation carried out at Braunton Burrows (Devon) by the War Department (Lands Branch) has now successfully stabilized a large area of dunes, and experimental studies of controlled sheep grazing on the higher *Spartina* marsh in the Bridgwater Bay Nature Reserve have shown that grazing favours succession towards a salting pasture in which sea marsh grass (*Puccinellia maritima*) is an important constituent, whereas, without grazing, reed (*Phragmites communis*) and sea club rush (*Scirpus maritimus*) become dominant. A climatological station has now been set up at the Dolgarrog Power Station, near the Coed Dolgarrog Reserve, Carnarvon, and

* The Nature Conservancy. Report of the Nature Conservancy for the year ended 30th September, 1959. Pp. vi+120+14 plates. (London: H.M. Stationery Office, 1959.) 6s. 6d. net.

climatological observatories, including recording of potential evaporation, have been started by officers of the Grouse Inquiry at two sites in Glen Esk.

There is much of scientific interest also in the work of the Conservancy's Scientific Advisory Services, and in this field it has now been agreed that inquiries on physical or engineering aspects of coastal defence shall be referred to the Hydraulics Research Station of the Department of Scientific and Industrial Research and that the latter station will refer requests for advice on biological aspects to the Nature Conservancy. Work is now proceeding on establishing water-level gauges at key points in several Reserves, and a further year's observations on the series of small controlled catchments in the Moor House Reserve have confirmed the tentative conclusion that when the peat moor is undrained, ungrazed and unburnt, the sponge effect of the peat is enhanced and the run-off retarded and rendered more even through the seasons. While the Conservancy is taking the opportunity, in co-operation with the Botanical Society of the British Isles, and the Department of Biology of the University College of North Staffordshire and local naturalists to obtain data on the effects of toxic chemicals on roadside verges in Staffordshire, it is taking any action necessary to conserve flora and fauna in Staffordshire from the effects of the County Council's spraying programme and continues to urge the Council to bring its spraying programme into line with the practice recommended by the Ministry of Transport's Circular. The Wild Life Panel of the Interdepartmental Advisory Committee on Toxic Chemicals in Agriculture has been set up and will be asked to prepare guidance for industry on the type of data about wildlife hazards required when the Advisory Committee is notified about a product. The intensive study of red grouse in Glen Esk, Angus, undertaken by the University of Aberdeen, indicates that the grouse almost certainly adjust their own population density, through territorial behaviour and the exclusion of surplus birds, chiefly at two periods of the year—first in late autumn and then early in the spring before the breeding season. Variations in breeding density from year to year appear to depend more on the condition of the heather than on annual variations of breeding success, and a start has been made on the measurement of heather productivity. The marking of grey seal pups continued in the Farne Islands, West Wales, and the north of Scotland, and much is being learned about the movements to and from the main breeding colonies, the length of life, and the mortality among young seals. A special grant has now been made to the Conservancy for research on grey seals in relation to fisheries.

The section of the report dealing with conservation records an increase in the number of National Nature Reserves from 70 to 80, while seven additional sites are managed as Local Nature Reserves with the advice of the Conservancy. Seven areas held for forestry purposes are similarly managed as Forest Nature Reserves with the advice of the Conservancy and two other areas enjoying statutory protection as wildfowl refuges have wardens through the support of the Conservancy. In Scotland, thirteen out of twenty-two National Nature Reserves have now been brought under comprehensive management plans approved by the Conservancy, and in England eighteen out of forty-two, and in Wales three out of sixteen. The variety of problems presented in managing the reserves is illustrated in the present report by fairly full accounts of the plans for the Cairngorms Reserve and the Cors Tregaron Reserve. In the latter, special attention is given to the cutting of peat, drainage and prevention of damage by fire.

The Conservancy's duties under its Charter have been fully recognized by the Secretary of State and provision made to strengthen its scientific and field staff to enable them to play their full part in informing and advising the Red Deer Commission, appointed under the Deer (Scotland) Act, 1959, on scientific aspects of its work. The Conservancy is also seeking to determine the pattern of migrating and wintering birds in the Bridgwater Bay Nature Reserve week by week and their daily and hourly movements between roosts and feeding areas, with a view of meeting objections made by local wildfowlers to the Conservancy's by-laws for this Reserve. This step illustrates one of the most important aspects of the Conservancy's work—the establishment of effective public relations. These are reviewed specially in this year's report, and the Conservancy affirms its view that the key to informing the general public about nature conservation must lie in the schools. This is a long-term approach and will need to be supplemented by other measures, particularly long-term, but the Conservancy is prepared to withstand temporary local unpopularity rather than take an easy course or accept a compromise which would jeopardize the maintenance for future generations of that part of the nation's scientific heritage with which it is entrusted. The Intelligence Unit established on May 4, 1959, by the Council for Nature should be of material assistance both in the provision of common services for biologists and naturalists and in solving such problems of public relations and public information.

SCIENTIFIC AND INDUSTRIAL RESEARCH IN NEW ZEALAND

THE report of the Department of Scientific and Industrial Research, New Zealand, for the year ended March 31, 1959, includes the Minister's Statement, the Council's report and that of the Secretary, together with the first annual report of the Ross Dependency Research Committee*. Lists of publications are given under departmental reports, and there are notes on the work of the incorporated research associations and on grant-aided research at the Canterbury Agricultural College, the Cawthron Institute, the Massey Agricultural College, the Seagrave Radio Research Station, University of Auckland, the Industrial Development Department and the Rolleston Radio Field Station, University of Canterbury. Early in the year an Atomic Energy Committee was appointed as a sub-committee of the Council to advise on the establishment and operation

* New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March, 1959. Pp. 112. (Wellington: Government Printer, 1959.)

of a nuclear research institute, towards the first stage of development of which the Government has already approved the expenditure of £240,000. Expenditure in 1958–59 was £1,658,149 gross, compared with £1,535,000 in 1957–58, the latter figure being 0·142 per cent of the national product, compared with 0·137 per cent in 1956–57 and 0·173 per cent in 1949–50. Major expenditures were: Dominion Laboratory, £191,305; Dominion Physical Laboratory, £184,887; Geophysics Division, £164,865; Grasslands Division, £101,145; Soil Bureau, £98,876; Geological Survey, £96,359; and Plant Diseases Division, £81,562. Grants to research and allied institutions totalled £165,282, including £32,200 to universities and agricultural colleges and £83,061 to research associations. The professional staff at March 31, 1959, was 390 compared with 383 in the previous year, with 435 technical (410), 175 clerical (165) and 65 other staff, but the Secretary points out that New Zealand employs fewer scientists in its major government research organizations than in 1952.

The Secretary's report reviews briefly work completed during the year. Facial eczema research of the Soil Bureau has established that *Sporidesmium bakeri* is a widespread saprophyte which colonizes dead material of a wide variety of plants and is a common invader of litter in pastures, and it appears there may be several strains of the fungus. The Grasslands Division is studying the environmental conditions favouring rapid growth and sporing of the fungus. The Plant Chemistry Division is studying the chemical composition of the lush autumn pasture responsible for 'hogget ill thrift' as part of a long-term study of the chemical factors which influence the quality of herbage for animal production, and the Grasslands Division has obtained encouraging results in the selection of parent plants to be used for the first nucleus seed supply of the new long-rotation rye-grass. Use of DDT and BHC, if applied at the appropriate time indicated by the life-history, has given promising results in control of adult Argentina stem weevil (*Hyperodes griseus* Hust) in rye-grasses, and a survey of two parasites liberated in the Nelson area against oak leaf miner (*Lithocolletis messaniella* Zell.) indicates that the winter parasite produces 85 per cent parasitism and promises to reduce damage by the leaf miner to insignificance.

Incorporation of 0·25 per cent dieldrin in glue for plywood, which has been recommended for protection against borer (*Anobium punctatum*), has become standard practice in the plywood industry. The search for a new apple rootstock to replace Northern Spy has now led to a new rootstock going into service, and much has also been done to reduce the virus infection in plum and apple root-stocks in nurseries and to assist the selection of virus-free budwood trees. Detailed studies of the freezing-point of pears have shown that this is likely to vary with different varieties and at different stages of ripeness and also from core to skin. The initial freezing-point of flesh near the core, the highest at which freezing can begin, is the temperature needed in practice by the cool-store engineer. As a result of a programme of breeding for resistance to disease initiated in 1952, improved varieties of cabbage, carrot, pole bean, glasshouse tomato and garden and field pea have already been released from the Division, and some 109 species, eight varieties and six hybrid weeds have been recorded, some now well-established and troublesome weeds, as additions to the weed flora of New Zealand, some sixteen species being toxic to livestock. Considerable progress is reported of studies on the response of the hop plant to environmental factors, especially light and temperature, and at the Tobacco Research Station small refinements have been demonstrated, the combined effect of which in yield and protection against black root-rot and mosaic can be considerable.

A survey by the Soil Bureau on mineral colloids revealed extensive deposits of low-grade aluminium ore, while wild-life research has included a study of the role of parasites in the natural control of rabbit populations and the inter-relations of cats, rats and petrels on the Little Barrier Island bird sanctuary. Forest surveys have shown that sufficient *Dacrydium biforme* is present in the Westland area to satisfy a market demand for manool for ambreinolid of up to 20 tons a year for 25 years. Operating conditions have been established for a process for the manufacture of hydrochloric acid for casein precipitation from sodium chloride by ion exchange resin. During the year the geothermal section, transferred to the Geophysics Division from the Dominion Physical Laboratory, has evaluated the heat escaping naturally and mapped the boundary of the Wairakei geothermal area, and theoretical studies and laboratory experiments are in progress to clarify the mechanics of fluid convection in permeable rocks and of the flow of steam–water mixtures from the rocks into a drill-hole.

Much of the effort of the Geophysics Division was directed to the programme of the International Geophysical Year, and the 1958 teams at Scott Base and Hallett Station had an extremely successful year. Results from the magnetic observatories at Apia (Western Samoa) and Amberley (New Zealand) and the ionospheric observations extending from Rarotonga (Cook Islands), through Godley Head (New Zealand) to the Antarctic on the phenomena observed with the Johnston Island nuclear explosions in August 1958 are still being collected and it is hoped that the study of the ionospheric and magnetic effects of the explosions will throw considerable light on the theory of magnetic storms and auroræ and the associated ionospheric disturbances. A systematic survey by the New Zealand Oceanographic Institute of the properties of the water of the Ross Sea, the animal life and the sediments from the east coast and the ice shelf to the 1,500 fathom line is providing a general regional picture of fundamental significance, and the abundance of minute and lowly organisms in the shallower waters supports the larger and better-known animals of Antarctica, the penguins, skuas, seals, and the killer and other whales. A fairly extensive study of the southern aurora was undertaken, and this is now to be continued in a few specific experiments, chiefly at Invercargill, but also at Scott Base and Hallett Station, at Campbell Island and in Central Otago.

An analogue has been built which will simulate yield in single-story structures, and the results obtained in tests with six of the severest earthquakes yet recorded indicate that if a structure is designed to yield at a seismic coefficient of 0·3, the permanent set obtained in a severe earthquake is of the order 1/16–1/4 in. between floor and ceilings. Two experimental solar heaters are being built at the Dominion Physical Laboratory and a thorough study has been undertaken of the propagation of very low frequency (16 kc./s.) waves over great distances. Results are now being obtained with the tritium technique in the

Division of Nuclear Science, and it should be possible to use this tracer to calculate the time for ground water to sink to depths at Wairakei and to be heated. The possibilities of radiocarbon as a tracer for studying the movements of water and gas underground in the thermal areas have also been investigated, and it has been shown that in the Wairakei area water takes probably considerably less than 40,000 years to travel from the surface to depths of 1,500 ft. Much assistance was given by the Geological Survey in the investigation and construction of large hydro-electric dams, while during the year electric resistance strain gauges were applied to a variety of problems, and the Applied Mathematics Laboratory and the Dominion Laboratory co-operated in investigations on the cause and prevention of outbreaks of fire in bales of greasy wool on board ship, and have shown that for a given air temperature there is a critical size for a volume of the material: a greater volume inevitably ignites. Further investigation of the lipids of red clover chloroplasts has shown that linolenic acid is accompanied by undecanoic acid, and the Wheat Research Institute has developed a rapid testing method—the Zeleny test—for baking quality.

METHODS FOR THE EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF SURFACE-ACTIVE COMPOUNDS

THE Society for Applied Bacteriology and the Microbiology Group of the Society of Chemical Industry held a joint meeting on January 13, with Mr. L. M. Miall in the chair, when a symposium on "Methods for the Evaluation of the Antibacterial Activity of Surface Active Compounds" was held.

The first speaker, Dr. J. G. Davis (London), reviewed technical aspects of the problem. Detergents differ in their bactericidal action; anionics and non-ionics have little or no action, but are more effective detergents than the cationics, which have powerful antibacterial action. The amphoteric detergents have not yet been extensively tested but claims of powerful antibacterial action have been made. Of the cationic detergents, only the quaternary ammonium compounds are important in practice. They have only found wide application since 1935, when their strong germicidal powers were first emphasized, although they had been prepared in 1913. They are suitable for use with foods, as they are effective in dilute solution and are of low toxicity. They have a high degree of persistence, but traces remaining on food and utensils are not of public health significance. Non-ionic detergents have no action against bacteria, but compounds of these detergents with iodine, the iodophors, are active and are becoming important in the United States. Some of the amphoteric detergents, the betaine derivatives, are really 'internal quaternaries'.

Dr. Davis said that test methods should be as near as possible to the actual conditions in which the substance tested is used in practice. Laboratory tests should only be regarded as sorting tests. There are many variable factors, for example, the test organism; most of the work has been done with cultures of public health significance, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Strep. faecalis*, *Escherischia coli*, *Salmonella typhosa* and *Pseudomonas pyocyanea*. The Gram-negative bacteria are usually more resistant than the Gram-positives, and *Pseudomonas pyocyanea* is usually the most resistant. For most test purposes, however, *Staphylococcus aureus* is the more suitable strain. Cultures maintained in the laboratory may be less resistant than freshly isolated types, and the method of cultivation may also affect the results. The number of cells is another variable, also whether or not they are clumped. Dr. Davis suggested an inoculum of 1 million cells, a convenient starting number when looking for 99·99 per cent kill, and thought it probably best to use normal laboratory culture media because the recovery of damaged cells was reported to be better than on special rich media.

In the Rideal-Walker test in particular, cell concentration is very important. Care is needed in examining tubes for growth, because low concentrations of quaternary ammonium compounds can cause turbidity in the medium. When testing quaternary ammonium compounds it is best to assume that a surface film is left on all the glass-ware, and to rinse it therefore with the test solution before doing the tests. Another difficulty is the increasingly powerful germicidal activity of slurries with increase in the amount of undissolved solid. The nature of the suspending liquid is also important, for example, the hardness of water can have very pronounced effects. The value of detergent sanitizers is often due largely to their detergent action; it is often sufficient to wash bacteria away. The alkalinity of a preparation may be of great value. The effect of organic matter on antibacterial action varies very greatly with the type of germicide. An addition of 4 per cent milk was suggested giving approximately 0·5 per cent solids, which is roughly equivalent to the amount of solids in dirty washing-up water.

In conclusion, Dr. Davis referred to the value of the Rideal-Walker test, which he said is the most important test in the history of disinfectant testing, but also the most misunderstood and abused. The Rideal-Walker test was originally devised for phenolic compounds; there is controversy on its applicability to quaternaries, and it is difficult to avoid commercial bias. The effect of quaternaries is largely due to their very great power of adsorption; in practical use there is no quenching of residual disinfectant with 'Lubrol W' or lecithin, which are used in laboratory tests. All buyers of disinfectants should receive adequate information on their potency, although it is not important to reveal the precise chemical composition. All manufacturers should declare the activity of the compound on at least two standard tests, which should be specified in detail.

In the discussion following the paper, it was remarked that *Staphylococci* are not necessarily more sensitive to quaternary ammonium compounds than the Gram-negatives, in fact some are more resistant than *E. coli*; the resistant *Staphylococci* usually come from hospitals. Whereas most quaternary ammonium compounds are ineffective against yeasts and moulds,

some are effective against *Candida albicans*. Generalizations are therefore unwise.

The second speaker, Dr. B. A. Newton (Cambridge), dealt with "The mechanism of the bacterial action of synthetic surface active compounds". There are two schools of thought on this problem. Some workers feel that the problem is solved, except for a few details. Others feel that the outstanding details are very important, and existing ideas need revision; the case for this was presented.

It is generally agreed that the action of surface-active compounds is bactericidal, not bacteriostatic. The kill is proportional to the concentration of surface-active agent, and there is no convincing evidence of reversal. Cells can be protected from this antibacterial action by suitable compounds added before, but not after, the treatment.

There are three main hypotheses as to the mechanisms of the antibacterial action: (1) there is a general and non-specific denaturation of cell protein; (2) specific detergent-sensitive enzymes are inhibited; and (3) the permeability of the bacteria is altered.

There are several approaches to the problem. It has been shown that it needs a higher concentration to affect the metabolism of the bacterial cell than to kill it. Lysis by both anionic and cationic detergents has been shown, and in 1942 Hotchkiss produced the first evidence of the leakage of nitrogen and phosphorus compounds into the medium without lysis. Many compounds are now known to be released; for example, glutamic acid can be rapidly and quantitatively released by cetyl trimethylammonium bromide. There is also appreciable binding of detergent by bacteria. It is calculated that the saturation level of adsorbed cetyl trimethylammonium bromide if in the form of a monolayer occupies about twelve times the area of the bacterial surface, whereas a bactericidal concentration corresponds to about half the surface area. Electrophoresis shows that adding detergent causes a reduction of the negative charge on the cells, and eventually a reversion to a positive charge. It is concluded that the reaction is therefore at or near the surface with a maximum depth of 100 Å. This evidence for surface action is supported by electron microscopy.

Since 1950, new techniques have permitted the isolation of cell walls of high purity. Beneath the cell wall, the existence of the protoplasmic membrane as an entity has been demonstrated. This membrane is a fragile structure, which is very sensitive to osmotic changes, and the cell wall gives it mechanical stabilization rather like the outer tube protecting the inner tube in a car tyre. There are two mechanisms of surface action: (1) penetration of the cell, resulting in lysis and (2) alteration of the structure of the cell wall, preventing its mechanical support of the cell. Cationics readily combine with isolated cell walls; anionics such as sodium lauryl sulphate can cause the complete lysis of cell walls of Gram-negative bacteria such as *E. coli*. This is due to the removal of lipoprotein from the cell walls. The reaction of cationics with the protoplasmic membrane has been demonstrated by Tomcsik and by Newton, and a reactivity sequence has been determined. There is evidence, then, for both mechanisms—an effect on the cell wall and on the membrane. Cationics and anionics may act in different ways; isolated membranes are completely dispersed by sodium lauryl sulphate, but 'Cetavlon' has no action. Cationics can cause small holes in the protoplasmic membranes (cytochrome c_3 is the largest molecule detected to leak through). Phospholipids have been suggested as a possible site for both cationics and anionics, but the visual changes (under the electron microscope) produced by the two types of detergents are quite different.

Ideas concerning the cell membrane have changed during the past few years. It is now known to be a dynamic structure, probably concerned with the permease system, cytochromes and protein synthesis. Interaction with detergents may therefore be more complex than at first thought. They may, for example, interfere with the maintenance of the membrane. The subject is still highly controversial, Dr. Newton concluded. In discussion the opinion was expressed that the anionics probably affect proteins rather than phospholipids. Some disagreement was evident about the lethal effect of quaternaries, some speakers claiming that they can be bacteriostatic. The possible importance of the effect of detergents on respiration was also mentioned.

Dr. Betty Hobbs (Colindale) described methods for measuring the practical efficiency of surface-active agents, with particular reference to the catering industry. For detergent efficiency, the difference in light transmission through soiled slides before and after controlled washing can be used. The type of soiling to get standardized results caused some difficulty. A wide range of substances were to be tested and soap and borax were used as standards; the soiling technique was required to show the maximum possible difference between these two substances. The best soil was a complex mixture devised by Hucker. With this, soiled slides after treatment with 0·3 per cent borax gave 10 per cent transmission, while 0·3 per cent soap flakes gave 95 per cent. On this test, soaps and household detergents were good, anionics alone were fair, and cationics and non-ionics gave poor results (household detergents are usually anionics plus inorganic materials).

The bactericidal power was measured by the reduction in bacterial numbers during 10-min. exposure; *Escherichia coli* Type I and coagulase positive *Staphylococci* (of a known food poisoning phage type) were used as test organisms. Best results were obtained with cationics plus inorganic salts, but some preparations professing to sterilize gave rather poor results. The fats of micro-organisms in dishwater were investigated, using an inoculum of paratyphoid bacteria and counting at intervals up to eighteen hours. When soiled dishes were washed in the water, the numbers generally rose, although they fell in one experiment. The effect of detergents was studied in a restaurant, with sampling every fifty or a hundred plates during washing up. Inorganic detergent plus hypochlorite was best, while inorganic material alone gave fair results sometimes.

When similar tests were done aboard a passenger ship, large numbers of fæcal *coli* were always found in the washing-up water.

The last speaker was Miss Christina M. Cousins (Shinfield), whose paper was entitled "The assessment of surface-active agents as disinfectants in milk production and processing". Most of the substances tested are quaternary ammonium compounds, etc., and preparations containing hypochlorite plus detergent. The use of iodophors has also been proposed. There is no evidence that the surface activity of detergents makes them effective disinfectants. Miss Cousins supported Dr. Davis in thinking that laboratory tests should be close to user conditions and give so far as possible the same results as practical

tests. No distinction between the various type of disinfectant is necessary. Hypochlorites were officially approved for use on dairy farms in 1949. The first quaternary ammonium compound was approved in 1957; its performance was compared with sodium hypochlorite plus alkaline detergent in field trials and in the Hoy can test. All the results were close to those obtained with the control hypochlorite, and no significant difference was detected. These tests also showed that there was a close relationship between field trials and the Hoy can test, which is now largely relied upon at the National Institute for Research in Dairying.

There is some difference between the usual farm cleaning and disinfecting methods in Great Britain and the United States. The two operations are usually done together in the former, but in the latter they are generally separate operations. Many different laboratory test methods have been suggested in recent years to evaluate germicidal preparations for dairy use. Most of the tests were devised for quaternary ammonium compounds, and measure the effect of the test material either on suspensions of bacteria or on soiled surfaces, which may or may not be inoculated with pure cultures of bacteria. Of the tests devised in the United States five were listed by Miss Cousins, three being suggested for disinfectants and two for sanitizers. One of the latter tests, Chambers' modification of the Webber and Black method, indicates that the use of 50 p.p.m. hypochlorite is satisfactory, and good correlation with practice is claimed.

The tests suggested in Britain include the Hoy can test, which will be discussed more fully below, two methods using steel strips, one using rubber liners, and one using stainless steel pipes. In general, it is found that bacterial cells on surfaces are less easily killed than those in suspension, and differences occur between the performance of different groups of compounds in the two types of tests. Quaternary ammonium compounds, for example, do not give good results in suspension tests, but on the surface film (as in the Hoy can test) the results are quite different. The question applicable to all methods is, Can they replace large-scale tests? The Hoy can test has been scaled down from the original ten gallon to five gallon cans. Test substances are compared with similar dilutions (use and 2/3 use) of hypochlorite. The cans are randomized, and the results are treated statistically. The cans are soiled with milk, drained and dried in a thermostatically controlled room. They are treated with test material for a determined time, quenched, rinsed with Ringers solution and squeegeed to remove surviving organisms, which are counted. If the methylene blue reduction time of the milk used for the test is greater than 30 min. or if the temperature is less than 72° F., the counts will be too low. It is desirable that the hypochlorite should not produce sterile cans, hence the use of the 2/3 dilution. Low counts can also be due to too rapid drying of the films, so the humidity of the air and its rate of movement is also important: the amount of milk which is left on the sides of the cans is small, probably about 3 ml.

A suspension test is also used at the National Institute for Research in Dairying for screening new substances, but it has only a limited value, as it does not correlate as well as the Hoy test with practical trials. Iodophors are not good on the can test, but satisfactory on the suspension test; 50 p.p.m. iodine in an iodophor is approximately equivalent to 300 p.p.m. of chlorine, but there is some suspicion that the iodophors cause staining. With quaternary ammonium compounds, 200–300 p.p.m. is satisfactory. The relative effects of quaternary ammonium compounds and detergents are not yet established.

For testing preparations for use on milk processing plant, the Hoy test can be used, but the increasing use of new cleansing methods raises new problems. The long-term effect of using detergent sterilizers is not yet known. Is there a build up of contamination in crevices, etc., on the plant? Work is going on to find an answer to this problem. Evaluation of antibacterial compounds cannot be separated from work on detergency; the final evaluation must be their performance in practice.

EAST AFRICAN AGRICULTURE AND FORESTRY

THE East African Agriculture and Forestry Research Organization is an inter-territorial research organization administered by and forming part of the East Africa High Commission. Research workers in other parts of the world will be interested in the annual report for 1958 recently published*.

Four main investigations are in progress on the water use of trees and crops and the effects of land use on stream flow and local climate. Details are given of the areas investigated and the changes which occur when natural regeneration is replaced by plantations. An integral part of these experiments has been a study of the estimation of total rainfall over restricted areas with fairly close distribution of rain gauges. This involved the use of tree-top platforms for measuring rainfall at the level of the forest canopy, and some exploratory measurements were undertaken to find the optimum size of forest clearings in which to expose a rain gauge a little above ground-level.

Application to a wide range of East African conditions of Penman's method of estimation of evaporation from other meteorological data has led to the construction of new tables for rapid computation of the evaporation estimate. In catchment area research and soil erosion studies it is important to be able to describe the storm characteristics of the rainfall. The quantity, time of day, duration and intensity may be estimated from autographic rain gauges. The detailed analysis of such data indicates considerable differences in pattern of rainfall in different catchment areas.

Work on estimation of irrigation requirements of *arabica* coffee have been continued and comparisons made between different types of evaporation tanks. Traditionally, shade trees are said to be beneficial to tea plantations, and experiments are in progress to elucidate the complex of factors concerned. The

* East Africa High Commission; East African Agriculture and Forestry Research Organization. Record of Research for the period January 1 to December 31, 1958. Pp. vii+109. (Nairobi: Government Printer, 1959.) Sh. 3/50.

greater ability of sorghum stomata to recover from severe drought, as compared with those of maize, was confirmed in the field. Thus, some of the well-known resistance to drought by this crop is probably due to the ability of its stomata to recover from severe water shortage. Studies of the behaviour of stomata during and after recovery from drought is being extended to other crop plants. The effect of soil drying on humus decomposition and mineralization of soil nitrogen has been further studied, and it has been found that addition of activated charcoal to a dry soil completely inhibited the flush of decomposition that normally occurs when a dry soil is moistened. This supports the hypothesis that drying a soil increases the amount of decomposable organic material going into solution, and further experiments have lent support to the idea.

Ecological work during the year was largely concerned with the determination of climatic types of vegetation in south Kenya. An account is given of twenty-one years work on *Cassava* breeding for resistance to mosaic and brown streak viruses. Projects on maize breeding include a study of resistance in maize to the virus of streak disease and maintenance of rust races and the pure lines of maize resistant to one or other of the known rust races so that, in the event of new races appearing in the field, genetic studies may be resumed. A *Sorghum* breeding research scheme has been inaugurated. The nematologist has completed a survey of plant parasitic nematodes of East Africa. Species of root-knot nematodes have been found in indigenous forest throughout East Africa and may constitute a problem for future development of crops in such areas. The Animal Husbandry Division is making a study of the nutritive properties of maize with the object of finding why maize is an unsatisfactory pig food and whether it would be economic to correct its detrimental properties. Results to date suggest that supplementary nicotinic acid would permit the effective use of high-maize rations.

E. C. Humphries

EXCAVATIONS AT LA VENTA, MEXICO

A RECENT publication by the Bureau of American Ethnology of the Smithsonian Institution entitled "Excavations at La Venta, Tabasco, 1955", represents an important contribution to Mexican archæology*. It should be studied in conjunction with previous Smithsonian reports which are quoted in the bibliography, particularly "La Venta, Tabasco. A Study of Olmec Ceramics and Art" (Bulletin 153 of the Bureau), also by Dr. P. Drucker.

The La Venta or Olmec Culture remains elusive both in its distribution and in its temporal range, but now we know a great deal more than we did about the type site, and for the first time we have a series of radiocarbon dates which give an idea when that particular site was occupied. The Olmec Culture has given rise to a notable art style, examples of which are found over a far wider area than the rather limited parts of the tropical forests of the Gulf Coast of southern Vera Cruz and Tabasco, where its chief monuments have been found. Broadly speaking, this art style is characterized by stone carvings and pottery figurines representing beings with puffy, loose-lipped 'baby faces', some of which take the form of colossal stone heads, and by highly stylized jaguar faces obviously related to the human ones. These are sufficiently prominent to provide strong evidence of jaguar worship, even without some remarkable features of La Venta which are mentioned below. One of the most important outliers of the style, where it chiefly takes the form of pottery figurines, is at Tlatilco on the outskirts of Mexico City, and this in its turn has relationships of another kind with sites of comparable age in Guatemala, Ecuador and Peru. In Peru, but not so far in the intervening area, evidence of a strong jaguar cult has been found, and it is tempting to relate it with the Olmec one, although its artistic representations are very different. This is enough to show the importance of the Olmec Culture, and how welcome is further information about it.

La Venta is a ceremonial site with few signs of ordinary dwellings. It occupies a ridge on an island surrounded by swamps, and was accurately planned about an axis running approximately north and south. The portion of it which has been investigated lies north of a large pyramid and consisted of a number of platform mounds disposed about two courts, of which the northern seems to have been the most important. Apart from a limited use of stone facing blocks, and some palisades made of rows of basaltic columns, the actual construction was carried out entirely in clays and sands of various colours, some of which at least must have been carried considerable distances.

In conformity with the usual pattern of Mexican ceremonial sites, at least four major reconstructions have been distinguished in each part of the site which has been investigated, and a most remarkable feature in connexion with each of these stages was the digging of enormous deep pits through the older structures, in which were placed what are interpreted as massive offerings consisting of pavements of slabs of varieties of metamorphic rock, loosely described as serpentine. The largest of these was 28 courses thick, and contained something of the order of 1,000 tons of rock. In at least two cases these underlay an even more remarkable mosaic jaguar mask measuring about 15 by 20 ft. of serpentine slabs set in clays and sands of various colours, and it is probable that more remain to be discovered. The facts that these were deliberately buried before a single rainy season had passed, and that none of the rocks of which they were made occurs within less than 60 km. from the site, and some perhaps more than 100 km., add to a most amazing phenomenon. Other features of the site were smaller offerings, not it seems connected with burials, some of which are related in position to the axis of the site; they include many groups of jadeite celts, and a most curious group of typical baby-faced sexless Olmec stone figurines which appear to be arranged to represent some ceremony.

* Smithsonian Institution: Bureau of American Ethnology. Bulletin No. 170: Excavations at La Venta, Tabasco, 1955. By Philip Drucker, Robert F. Heizer and Robert J. Squier. With Appendixes by Jonas E. Gullberg, G. H. Curtis and A. S. Leopold. Pp. viii+312+63 plates. (Washington, D.C.: Government Printing Office, 1959.)

The nine radiocarbon dates contain some minor inconsistencies, but taken as a whole they justify the conclusion of the authors that the time covered by the four building periods is from about 800 to 400 B.C. This is an important new piece of evidence to add to that which has been accumulating in recent years for the existence of elaborate ceremonial structures in the first millenium B.C., that is to say before the Classic Period in the first millenium A.D. to which belong the famous ceremonial centres of the lowland Maya and those at Teotihuacan and Monte Alban. Both stages imply the disposal of much labour by a ruling class, witness the carriage of 1,000 tons of stone for more than 60 km. without wheeled transport or pack animals, for a single ritual deposit at La Venta. The chief difference seems to be that clay rather than stone and lime plaster was used for constructional purposes in the pre-Classic sites. The report also gives powerful support to those who believe the Olmec to have preceded the lowland Maya and other high cultures of this part of the American continent.

G. H. S. BUSHNELL

MISTLETOE IN AUSTRALIA

MISTLETOE has never been a serious pest in Britain. The Australian species, however, are different and cause extensive losses (C.S.I.R.O., *Rural Res.*, **29**; 1959). They do not even find a use as Christmas decoration. Only in Tasmania is the parasite not a problem, but why this is so when it occurs widely on the mainland is not known. In the other States, and especially in New South Wales and Queensland, mistletoe damage is so extensive and so costly that it constitutes a problem of national importance.

Areas may be found in which practically every tree has been parasitized. Many of them die, and those that do not produce less timber. One worker has estimated that when 38 per cent of the crown of the host tree has been displaced by mistletoe, wood production is down as much as 55 per cent.

The widespread, outright killing of trees in water catchment areas has led to soil erosion, silting, and higher flood-levels in wet seasons. In agriculture, mistletoe is a nuisance, as it often kills shade trees.

Forestry departments have found that the incidence of mistletoe attack has been increasing over recent years. For example, records maintained by the Commonwealth Forestry and Timber Bureau on a number of small plots at Kowen show that between 1952 and 1955 the infestation increased from 75 to 79 per cent a year.

Eradication based on amputation of infested branches or felling badly infested trees is much too expensive for large-scale operations. Certain sprays are efficient killers; but it is often difficult to reach all the mistletoe on an affected tree with spray equipment. Biological control methods, using insects or pathogens, hold little hope of success. In 1937, the Forestry Department of Queensland found that possums had a decided liking for mistletoe and apparently preferred it to almost any other type of food. It is possible that before the coming of the white man, these and other leaf-eating animals exerted a strong control on mistletoe. It is impracticable under present conditions to restore the possum population to its former level. Clearing for settlement, resulting in more light and greater numbers of birds around the trees, has probably increased the spread of mistletoe: it seems to grow best where the light is good, and birds are the chief means of spreading the seeds, which stick to their beaks or pass through with their droppings.

Chemicals seemed to offer the only effective means of control, and, in 1948, Mr. G. C. Greenham, of the Commonwealth Scientific and Industrial Research Organization Division of Plant Industry, began experiments to see whether it would be possible and practical to control the parasite by a selective poison injected into the trunk of the host tree. The work has entailed exhaustive trial and error because of the close physiological association between the host and parasite—a poison that would kill the mistletoe might also be expected to kill or at least seriously damage the tree.

The first poison tried was the selective phytocide, copper sulphate. This gave fair results, but was not good enough, because usually it did not kill more than half the mistletoe. The next step in the investigation was to obtain information about the way in which substances are moved or translocated throughout a tree. This was done by using radioactive isotopes. Supplies of radioactive iron, cobalt and zinc salts were used in the first botanical experiment with radioactive isotopes in Australia.

The third step was to try out the hormone selective weed-killers. So far, about a dozen of these have been checked; the triethanolamine salt of 2,4-*D* was found to be outstanding in that it killed or seriously injured a high proportion of the mistletoe without harming the host eucalyptus.

Other hormone-type compounds and mixtures of them are being tested, and some, including various forms of 2,4-*D*, hold considerable promise. The hydrazine salt of 2,4-*D* is being exhaustively tested at present because preliminary trials have shown it to be at least as good as the triethanolamine salt and to have a faster effect.

Mistletoe dies up to two years after the tree has been injected, and the final assessment of the effectiveness of the treatment should be postponed until at least two years have elapsed. Mistletoe surviving after this time may be subjected to the treatment again without harming the host tree, which does not become sensitized to 2,4-*D* as a result of earlier dosing. Nor does the mistletoe become resistant, and the second treatment will kill about 50 per cent of the remaining parasites. Because of the high rate of re-infestation in some areas, it would be unrealistic to aim at complete eradication. Since the first treatment may be expected to kill 70–90 per cent of the parasites, it is more practical to re-treat the trees every five years.

Information is now available concerning the effect of the treatment on eight species of eucalyptus. Reasonably good results have been obtained for seven of the eight species, and this suggests that the treatment may be suitable for destroying mistletoe on a wide range of eucalyptus hosts.

FUNCTION OF THE TWO CYTOCHROME COMPONENTS IN CHLOROPLASTS: A WORKING HYPOTHESIS

By Dr. R. HILL, F.R.S., and Dr. FAY BENDALL
Department of Biochemistry, University of Cambridge

THE conversion of light into a form of chemically available energy by chloroplasts *in vitro* can be observed in two ways: by the reduction of a hydrogen acceptor with the production of a stoichiometric equivalent of molecular oxygen[1] or by the formation of adenosine triphosphate from inorganic phosphate in the presence of adenosine diphosphate[2].

In the green plant, coenzyme II can be regarded as the hydrogen acceptor for the complete photochemical system of the chloroplast. The reduction of coenzyme II is mediated by a soluble protein[3], which can be separated from the green insoluble or particulate system containing both chlorophyll and cytochrome components. The phosphorylation reaction is a property of the insoluble fraction, since the coenzyme and soluble protein can be replaced by other hydrogen acceptors. This is shown diagrammatically in Fig. 1, the rectangle representing the green insoluble part of the chloroplast. The four equivalents of hydrogen can be regarded as being derived from water (they might possibly arise from the water removed when phosphorylation of adenosine diphosphate takes place). Removal of the hydrogen by an acceptor system liberates molecular oxygen and, for each molecule liberated, two molecules of inorganic phosphate can be esterified.

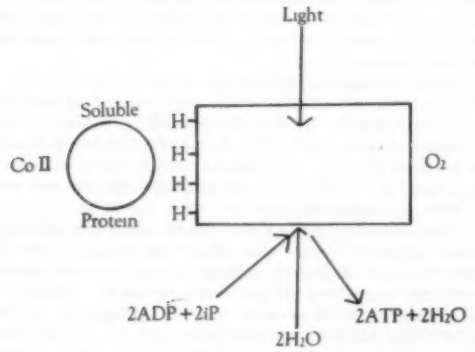

Fig. 1. ADP = adenosine diphosphate; ATP = adenosine triphosphate; iP = inorganic phosphate

The reduction process can be symbolized by the transfer of hydrogen (or electrons) from water to the hydrogen acceptor, the overall reactions per equivalent of hydrogen transferred being usually represented as follows:

$$\begin{aligned} H_2O + X + Y &\rightarrow XH + YOH \\ XH &\rightarrow X + (H) \\ YOH &\rightarrow Y + \tfrac{1}{2}H_2O + \tfrac{1}{4}O_2 \\ \hline \tfrac{1}{2}H_2O &\rightarrow (H) + \tfrac{1}{4}O_2 \end{aligned} \qquad (1)$$

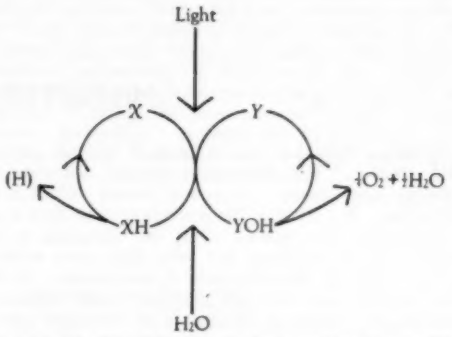

Fig. 2. *X* and *Y* are the hypothetical first acceptors of the products of the photolysis of water, forming respectively the reduced product *XH* and the oxidized product *YOH*

It can also be represented as a diagram as shown in Fig. 2. The symbols *X* and *Y* represent a postulated mechanism which separates the oxidized from the reduced part, (H) represents a source of active hydrogen transfer in a thermochemical sense. This representation corresponds with the reversal of a 'terminal oxidase' in respiration, the reaction characteristic of a mitochondrion or its fragments. In the mitochondrion, the transfer of hydrogen is from a hydrogen donor to molecular oxygen to give water. This can be represented by reversing all the arrows in (1) or in Fig. 2, and this generally will represent a thermochemical reaction taking place in the dark.

From this representation we are led to generalize that light absorbed by the pigments can transfer hydrogen against the thermochemical gradient.

In the respiratory chain mechanism of mitochondria the transfer of hydrogen (or electrons) is mediated in a stepwise way by the flavoprotein and cytochrome system. The transfer of the hydrogen is found to be coupled with phosphorylation reactions which appear to correspond with the stepwise transfer of the hydrogen (or electrons). Part of the energy at each step derived from a thermochemical oxidation process is conserved in the form of adenosine triphosphate.

In the chloroplast we find two cytochromes, *f* and b_6, which appear to be similar to the cytochromes *c* and *b* of the mitochondrial system. But instead of the terminal oxidase (associated with cytochrome *a* components), there is a relatively massive amount of chlorophylls which absorb the light[5]. To explain the formation of adenosine triphosphate from inorganic phosphate and adenosine diphosphate by the illuminated chloroplast, the very simple hypothesis would be that *XH* and *YOH* react through the two cytochromes to give water again, concurrently with the phosphorylation reaction.

However, this simple hypothesis, involving one 'light-driven reaction', does not at present seem to

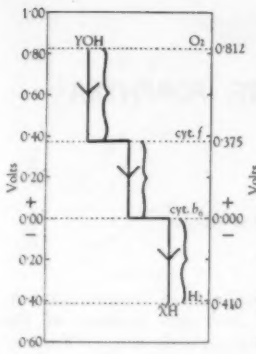

Fig. 3. Symbols as in Fig. 2

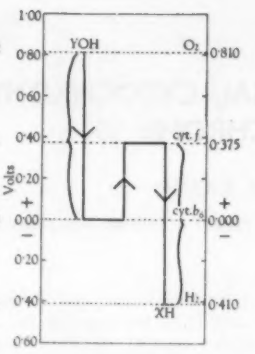

Fig. 4. Symbols as in Fig. 2

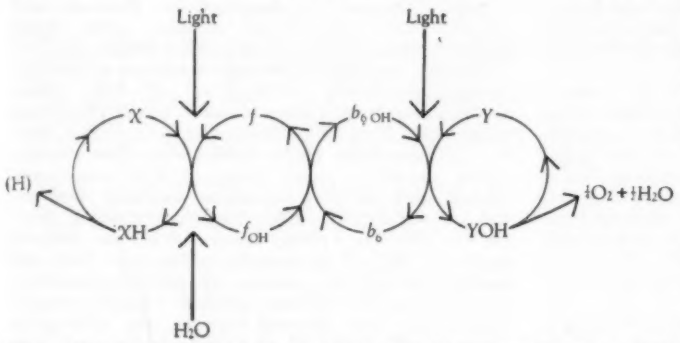

Fig. 5. *X*, *Y*, *X*H and *Y*OH as in Fig. 2. *f* and b_6 represent ferrous and f_{OH} and b_{6OH} ferric or oxidized cytochromes

fit in with experiment. If the hydrogen transfer, represented as recombination of hydrogen and hydroxyl to give water again, is coupled with the phosphorylation, we should expect the addition of phosphate acceptor (adenosine diphosphate) and inorganic phosphate to diminish the observable rate of reduction of an added hydrogen acceptor and also of the corresponding observable rate of oxygen production. If the coupling was not obligate, then at the best we should expect no observable effect. But, on the contrary, Arnon's group[6] discovered that, in the presence of adenosine diphosphate, inorganic phosphate and magnesium ions, the rate of reduction of ferricyanide and the rate of production of oxygen were significantly increased and even doubled.

Thus the chloroplast system would appear to act by reversing a stepwise hydrogen transfer, as suggested by Davenport and Hill[7]. The phosphorylation reaction in light would then be regarded as 'reductive'[8], for the hydrogen (or electron) transfer is against the thermochemical gradient. This, if it is supposed that cytochromes b_6 and *f* are concerned, would involve three separate light-driven reactions. This scheme is shown as a potential diagram in Fig. 3. The downward arrows show hydrogen transfer against the thermochemical gradient and the brackets show the effect of each of three light-driven reactions.

The postulation of two light-driven steps, rather than three, would be in better accord with present experimental results. In this case oxidized cytochrome b_6 would have to be reduced by *Y* to give *Y*OH and cytochrome *f* would have to be oxidized by *X* to give *X*H. The reaction between cytochromes *f* and b_6 would then be a thermochemical process and quite analogous with a hydrogen transfer step characteristic of the mitochondrion. This is shown as a potential diagram in Fig. 4, and by the diagram in Fig. 5 to correspond with Fig. 2. In Fig. 5, *f* and b_6 represent ferrous and f_{OH} and b_{6OH} ferric or oxidized cytochromes. If the reaction between *f* and b_6 could be inhibited, we should expect from this hypothesis that *f* would become oxidized and that b_6 would tend to be reduced in light. In bacterial chromatophores it might be possible to test this hypothesis relating c_2 (corresponding with *f*) with the cytochrome b_6 component[9]. In the green plant there is no evidence so far as we know at present which would indicate a phosphorylation step between *f* and b_6.

In the scheme put forward by Arnon[10], the proposed properties of the cytochromes do not correspond with the observed properties of cytochromes *f* and b_6. They would have to have oxidation-reduction potentials more positive than + 0·81 V. at *p*H 7. The present hypotheses are consistent with the data regarding the cytochrome components, but the nature of *X* and *Y* is still obscure. We might perhaps assume for the moment that both *X* and *Y* are forms of a part of chlorophyll. But the present experimental evidence taken as a whole could not consistently support such an assumption, particularly in the case of *Y*. It must therefore be emphasized that Fig. 5 is only a hypothesis in barest outline; for example, the reaction between the two cytochrome components is not likely to be a direct process, as shown.

The reconstituted chloroplast system of Vishniac[11] would correspond with the left-hand half of the system in Fig. 5, being active while the right-hand half, responsible for oxygen production, is inactive. With the reduction of coenzyme by the partially inactive system, a corresponding amount of some hydrogen donor would have to be oxidized.

In summary, it can be stated that if the cytochrome in chloroplasts is directly concerned in hydrogen (or electron) transfer, the system would require more than one light-driven reaction to act in opposition to the thermochemical gradient.

This communication deals with work forming part of a programme supported by the Agricultural Research Council.

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CRYSTALLIZATION OF AN ALGAL CYTOCHROME, *PORPHYRA TENERA*-CYTOCHROME 553

By Dr. SAKAE KATOH

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THE apparently ubiquitous occurrence in algae of a group of cytochromes showing absorption at 552–3 mμ and a remarkably high level of oxidation-reduction potential at about 300 mV. has already been described[1] in the course of this series of investigations. Subsequent efforts to purify the hæmoprotein of this group have finally resulted in crystallization of one of them, which will be named *Porphyra tenera*-cytochrome 553.

The thalli of a red alga, *Porphyra tenera*, which is cultivated for food in the so-called 'Nori-field' (algal farm) of Tokyo Bay, served as the starting material. Fresh thalli were rapidly frozen at − 40° and stored at − 20°. The first step of the extraction procedure was essentially the same as that previously described by Yakushiji[2], who, as early as 1935, identified and partially purified this cytochrome. The frozen thalli were thawed by warming in pure water at 30° (1 litre per kgm. thalli), and allowed to stand at room temperature (15–20°) for 48 hr.

By this time, most of the cytochrome was found to have diffused into the surrounding medium. All the subsequent procedures of purification were carried out at room temperature. The crude extract thus obtained was strained through cloth and to the solution was added 450 gm. per litre of ammonium sulphate with stirring. The precipitate was collected by centrifugation and the cytochrome included in the sediment was further extracted three times as follows. The sediment was dissolved in a sufficient amount of water, to which 400 gm. of ammonium sulphate per litre were added with stirring, and the precipitate formed was discarded by centrifugation. To the combined supernatant, 300 gm. of ammonium sulphate per litre were added and the mixture was left for 4 hr. or longer. The precipitate formed was collected by centrifugation and dissolved in 200 ml. of pure water, with the addition of 25 ml. of 1 per cent rivanol solution in several portions. After removal of the precipitate by centrifugation, 175 gm. of ammonium sulphate were added to the supernatant. The sediment formed was collected, dissolved in 50 ml. of pure water and dialysed against pure water for 24 hr. In order to remove the rivanol remaining in the dialysate, a thick suspension of cation exchanger, '*XE* 64' (H^+-form), was added until the yellow colour of the detergent was no longer observed in the supernatant on centrifugation. At this stage of purification, most of the phycobilins and viscous substances in the original extract had been removed and a clear pink solution of the cytochrome was obtained. This solution, when treated with ascorbic acid to reduce the cytochrome, gave a 'purity index' (E 553/E 270) of 0·1, a value which was defined by the ratio of extinction at 553 mμ to that at 270 mμ. Cation-exchange resin, which has been successfully used by Hagihara *et al.*[3] in the crystallization of cytochrome *c* from various sources, could not be applied in this case because of the acidic nature of the algal cytochrome. Further purification of the cytochrome was therefore carried out by using an anion adsorbent, diethylaminoethyl-cellulose prepared as described by Peterson and Sober[4]. The cytochrome solution, after being dialysed against 0·025 *M* phosphate buffer (*p*H 7·0) for 24 hr., was passed through a column of diethyl-aminoethyl-cellulose buffered at *p*H 7·0. After washing the charged column with 0·025 *M* phosphate buffer (*p*H 7·0), the cytochrome was eluted with 0·1 *M* phosphate buffer (*p*H 7·0). The effluent, which showed a purity index of 0·55, was again fractionated with ammonium sulphate between 0·6–1·0 saturation. The fraction containing cytochrome (showing a purity index of 0·70) was dialysed against 0·025 *M* phosphate buffer (*p*H 7·0) and passed through a column of diethylaminoethyl-cellulose equilibrated with 0·025 *M* phosphate buffer (*p*H 7·0). The charged column was thoroughly washed with 0·025 *M* phosphate buffer (*p*H 7·0). When the concentration of the washing solution was raised to 0·05 *M*, a small amount of an orange-coloured pigment began to run down from a deep red zone of cytochrome at the top of the column. This orange pigment was found to be reduced with hydrosulphite, showing then absorption at 550 mμ. This pigment could not be reduced with ascorbic acid, indicating that the substance is different from cytochrome 553. After removal of the orange pigment, the cytochrome was eluted with 0·1 *M* phosphate buffer (*p*H 7·0). The eluate, which showed a purity

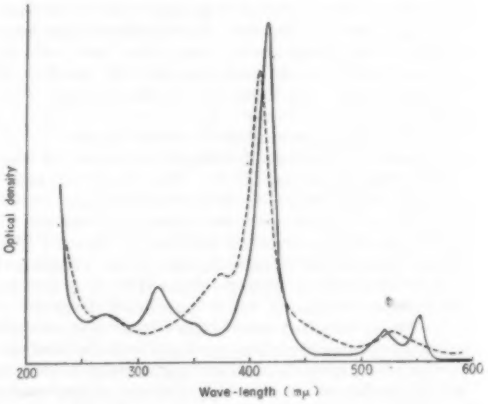

Fig. 1. Absorption spectra of crystalline *Porphyra tenera*-cytochrome 553. Solid line, reduced form; broken line, oxidized form (the cytochrome was oxidized by dialysing against 0·005 *M* ferricyanide for 12 hr.)

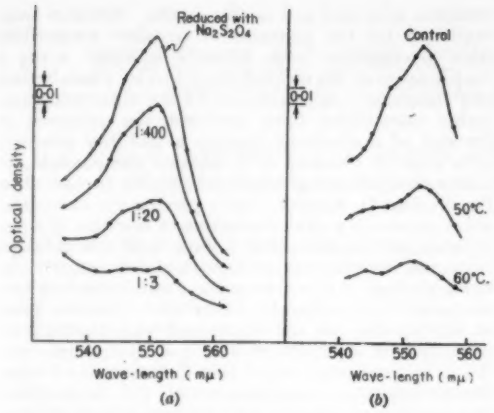

Fig. 2*a*. Spectra of α-band of *Porphyra tenera*-cytochrome 553 at various levels of oxidation-reduction potential. The mixture initially contained: 1·0 ml. of 0·1 *M* ferrocyanide, 1·0 ml. of phosphate buffer (*p*H 7·0), cytochrome solution and pure water to make a final volume of 2·8 ml. To the mixture, various amounts of 0·1 *M* ferricyanide were afterwards added and the absorption spectrum of the α-band was measured spectrophotometrically, each time after 5 min. for equilibration. The numbers under each curve represent the ratio of ferri- to ferro-cyanide

Fig. 2*b*. Spectra of α-band of *Porphyra tenera*-cytochrome 553 after heat-treatment. Cytochrome in 0·033 *M* of phosphate buffer (*p*H 7·0) was kept at the temperature indicated for 5 min., and after rapid cooling the absorption of the α-band was measured

index of 1·0, was saturated with ammonium sulphate and the precipitate formed was collected by thorough centrifugation. The sediment was dissolved in a minimum quantity of 0·5 *M* phosphate buffer (*p*H 7·0) and fine crystals of ammonium sulphate were added until the solution became slightly turbid. When the solution was allowed to stand for 24 hr. or longer, small needle-like crystals of the cytochrome appeared. The crystals were collected by centrifugation and dissolved in a minimum quantity of 0·5 *M* phosphate buffer (*p*H 7·0) and the recrystallization was carried out by the same procedure as described above. The yield of crystalline cytochrome was about 200 mgm. from 15 kgm. of frozen thalli.

The absorption spectra of the crystalline *Porphyra tenera*-cytochrome are presented in Fig. 1. Absorption maxima in the reduced form lie at 553 (549), 521, 417 (350), 317 and 272 mμ; in the oxidized form, at 565, 525, 408 (380) and 272 mμ; numbers in parentheses representing the shoulders. The ratios of extinction at the wave-lengths indicated were as follows: E 553/E 270, 1·04; E 417/E 553, 6·9; E 521/E 553, 0·67.

In common with other members of this group of cytochromes thus far studied in various algal materials (for example, *Grateloupia elliptica* and *Meristotheca papulosa*; unpublished work), a shoulder was consistently found to exist on the blue side of the α-band of the crystalline *Porphyra tenera*-cytochrome. This shoulder had also been noticed in the earlier study of *Porphyra* cytochrome by Okunuki and Yakushiji[5], who considered it to be due to a complex of the main cytochrome component (553 mμ) with cytochrome *c* (550 mμ). However, when the purified sample was subjected to oxido-reduction using a ferri- and ferro-cyanide mixture as oxidation-reduction buffer, the relation of the shoulder to the main peak of the α-band remained the same at any level of oxidation-reduction potential (see Fig. 2*a*). This eliminates the possibility that the α-band of the cytochrome is of a composite nature due to the existence of two pigment components having different oxidation-reduction potentials. Heat-treatment of the cytochrome solution also resulted in a parallel disappearance of the shoulder and the main peak of the α-band as illustrated in Fig. 2*b*. These facts suggest that the observed asymmetry of the α-band is an intrinsic property of this cytochrome, and is not due to the existence of an admixture.

I acknowledge the continuous interest and advice of Prof. H. Tamiya during this work. I also thank Prof. A. Takamiya for his encouragement and valuable suggestions throughout this work; Prof. M. Arasaki, for his assistance in procuring the fresh algal material; and the Nihon Reizo Co. for permission to use the facilities of their cold-storage plant; and Prof. K. Okunuki, University of Osaka, and Prof. E. Yakushiji, Toho University, for advice concerning the purification procedure.

A personal communication from Prof. Okunuki states that the crystallization of the same hæmoprotein from the same algal source has recently been achieved also in his laboratory.

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EXCRETION OF NICOTINIC ACID AND NICOTINIC ACID ADENINE DINUCLEOTIDE BY BIOTIN-DEFICIENT YEAST

By Dr. ANTHONY H. ROSE
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IT has been shown by Rose and Nickerson[1] that excretion of nicotinic acid by a number of biotin-requiring yeasts is either initiated or greatly stimulated when these organisms are grown in media containing sub-optimal concentrations of D-biotin. Although only a limited range of biotin concentrations was examined, the amounts of nicotinic acid excreted were always greatest in media in which the amount of biotin present was very small. We did not, however, ascertain the form in which nicotinic acid was excreted, the activity of culture filtrates being expressed simply as m.μgm./ml. 'nicotinic acid'. A detailed examination of the effect of varying concentrations of biotin on 'nicotinic

acid' excretion by a strain of *Saccharomyces cerevisiae*, together with an investigation of the form in which the vitamin appears in the growth medium, has recently been undertaken by me in this laboratory; the results of these studies form the subject of the present article.

Filtrates from cultures of the yeast grown in media containing an optimal concentration of biotin (0·20 m.μgm./ml.) were devoid of 'nicotinic acid' activity. But reduction in the biotin concentration evoked a marked excretion of 'nicotinic acid' by the yeast, with the vitamin content in filtrates from 3-, 5-, 7-, and 9-day old cultures reaching a maximum when the medium contained 0·005–0·010 m.μgm./ml. biotin. Excreting power, expressed as the quantity of 'nicotinic acid' excreted by 1 mgm. dry weight of yeast, was greatest in a medium containing 0·005 m.μgm./ml. biotin. The small amount of growth in media completely lacking in biotin was also accompanied by the appearance in the culture fluid of 'nicotinic acid', the amounts being small in 3- and 5-day old cultures, but increasing to 80 m.μgm./ml. after 7 days incubation. Excretion of 'nicotinic acid' in media containing more than 0·01 m.μgm./ml. biotin decreased markedly as the biotin concentration was raised, the vitamin being undetectable in culture filtrates from media containing more than 0·10 m.μgm./ml. biotin.

The strain of *Candida pseudotropicalis* that was used in the microbiological assay of 'nicotinic acid'[2] responded identically to both nicotinic acid and its amide, but diphosphopyridine nucleotide, sterilized by Seitz filtration and added aseptically to basal medium, did not elicit a detectable growth response from the organism, except in high concentrations (greater than 0·10 μgm./ml. nicotinic acid equivalent). When filtrates from 7-day old cultures of *S. cerevisiae*, grown in media containing 0·01 m.μgm./ml. biotin, were assayed by adding portions aseptically to sterile assay medium, the 'nicotinic acid' content of the filtrates appeared to be lower than when assayed using the standard procedure. This led to the suggestion that the vitamin existed in the filtrates both in a free form (as nicotinic acid or nicotinamide) and as one or more heat-labile combined forms, possibly pyridine nucleotides, which were broken down to nicotinic acid or nicotinamide when the filtrates were autoclaved momentarily at 10 lb./sq. in. with assay medium. Filtrates that had been hydrolysed with 0·1 *N* hydrochloric acid or 0·1 *N* sodium hydroxide did not show any further increase in activity. Approximately equal amounts (*c.* 100 m.μgm./ml.) of free and combined vitamin were present in filtrates from the above 7-day old cultures. In culture filtrates of the same age from media containing higher concentrations of biotin, the proportions of free and combined nicotinic acid remained roughly equal, although the total amounts excreted fell sharply with increasing biotin concentrations. The small amounts of 'nicotinic acid' excreted in biotin-free media were found to be present predominantly in the free form.

When filtrates containing both free and combined nicotinic acid were examined chromatographically[3], the presence of nicotinic acid, but not nicotinamide, was detected after placing the chromatogram paper over cyanogen bromide and spraying with 2 per cent *p*-amino benzoic acid[4]. Nor could nicotinamide be detected in filtrates that had been autoclaved momentarily at 10 lb./sq. in., showing that the vitamin was present in the combined form as nicotinic acid and not as the amide. Filtrates were examined for the presence of pyridine nucleotides after precipitation with mercuric acetate[5], using a modification of the method described by Chamberlain and Rainbow[6]. Examination of the chromatograms under ultra-violet light revealed the presence in filtrates of a substance having a mobility identical with that of nicotinic acid adenine dinucleotide, an authentic specimen of which was kindly furnished by Dr. Nathan O. Kaplan. After placing the chromatogram papers in a tank containing a mixture of equal volumes of methyl ethyl ketone and concentrated ammonia, and then examining again under ultra-violet light, this spot did not fluoresce, thus confirming that it was not a nicotinamide nucleotide[4]. Further proof of the identity of the compound was obtained on hydrolysis of a portion (0·5 ml.) of concentrate with 0·1 *N* hydrochloric acid at 100° C. for 10 min.[3], when chromatographic examination of the hydrolysate showed the presence, in addition to residual nicotinic acid adenine dinucleotide, of adenine, nicotinic acid, and of a substance (presumably nicotinic acid mononucleotide) having an R_F of 0·24. A similar chromatographic pattern was obtained on examination of a standard solution of nicotinic acid adenine dinucleotide after hydrolysis with hydrochloric acid. Neither nicotinic acid adenine dinucleotide nor nicotinic acid mononucleotide could be detected in concentrates prepared from filtrates that had been autoclaved momentarily at 10 lb./sq. in.

These results suggest that the biosynthesis of pyridine nucleotides in *S. cerevisiae* is severely impaired when the yeast is grown in the presence of sub-optimal concentrations of biotin, for under these conditions nicotinic acid and nicotinic acid adenine dinucleotide accumulate in the culture medium. Evidence has recently been obtained in several laboratories showing that, in a variety of living organisms, synthesis of diphosphopyridine nucleotide from nicotinic acid proceeds via the formation of nicotinic acid mononucleotide and nicotinic acid adenine dinucleotide[3,7]. The absence from yeast culture filtrates of nicotinamide, either free or in a combined form, is therefore particularly noteworthy, for it gives support to the theory that pyridine nucleotides are not synthesized directly from this amide[8]. However, the precise role of biotin in the biosynthesis of pyridine nucleotides in yeast cannot be inferred from these results. Accumulation of nicotinic acid and nicotinic acid adenine dinucleotide suggests that the further metabolism of these compounds, into nicotinic acid mononucleotide and diphosphopyridine nucleotide respectively, is blocked under conditions of biotin deficiency. But whether biotin functions coenzymatically in these reactions, or whether it acts indirectly as in synthesis of other reactants or of the enzymes concerned, is open to speculation. Work at present being carried out in this laboratory will, it is hoped, help to clarify our understanding of the part played by biotin in the biosynthesis of pyridine nucleotides in *S. cerevisiae*.

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INFLUENCE OF GEOLOGICAL FEATURES ON VERY LOW FREQUENCY GEOMAGNETIC FLUCTUATIONS

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THE Pacific Naval Laboratory investigation of the natural geomagnetic background during the past few years was at first confined to the frequency-range between 0·001 and 0·1 c.p.s. This has been intermittently recorded by frequency modulation on magnetic tape at 0·15 in./sec. monitored by Esterline-Angus or Brush strip chart recorders.

Throughout we have had in view the need for simultaneous records of the field fluctuations in the vertical (Z), and geographical north–south (X) and east–west (Y) directions. Our hope has been that simultaneous records of these fluctuating elements at stations separated by large and small distances would give clues to the origins and causes of these fluctuations.

The detectors were first set up at a base station at Albert Head, on the sea coast some four miles from the Pacific Naval Laboratory. It was soon found[1] that there was a considerable similarity between the X and Z records. This was especially true of the 'daytime' pulsations of period 15–30 sec., and it was observed that the fluctuating vector pointed more directly vertical the higher the frequency.

During the past two years records have been made simultaneously at the base station and at field stations set up in turn at William Head, four miles away from it and on the shore of the Strait of Juan de Fuca; at Bear Creek, 27 miles away but only 9 miles from the sea and in rough country; at Summerland, 166 miles away and east of the Coastal Range; near Ralston, 515 miles away in the Canadian Prairies; at Borrego Springs, in Southern California, 960 miles away; and again near Ralston, where two closely spaced stations were occupied simultaneously. (The distances are given in nautical miles.)

For all these operations except the last, the ratio of the amplitudes of the fluctuations in the X, Y and Z directions relative to those at Albert Head have been measured. Only those groups of fluctuations were used which could be recognized on the Esterline-Angus record to correspond at both stations. The significant characteristic of these ratios is that, for each directional component, the ratio often varies with the frequency of the signal background. Especially is this true of the vertical force (Z) fluctuations where, at each field station, the ratio becomes smaller at the higher frequencies. We attribute this chiefly to the circumstance that the local horizontal conductivity gradient is large at Albert Head, causing enhanced vertical magnetic field fluctuations at the higher frequencies. The ratio is especially small at Ralston for the higher frequencies (Fig. 1).

More recently, a modification of the equipment has enabled us to record higher frequencies, and it is now clear that there are some occasions when frequencies between 0·2 and 3 c.p.s. occur and can have a peak-to-peak amplitude approaching one gamma ($1\gamma = 10^{-5}$ oersted) sometimes even during magnetically quiet periods. (Normally, during a magnetic storm the recorded amplitudes become greater and one observes higher frequencies which rise to 3 or more c.p.s. in great storms.) In otherwise quiet magnetic conditions these high-frequency fluctuations of 0·2–3 c.p.s. may last 1 hr. or so and be preceded or followed by intermittent occurrence of the signals, as if we had to deal with occasional transmission windows between the source and the Earth. Often the records look as if a source of wide-band energy was filtered to allow only two narrow bands to reach the Earth (these have been called 'pearls'). Fig. 2 shows the intermittent type recurring at a constant interval of about 2 min. and Fig. 3 the more common development of 'pearls'.

'Pearls' of frequency about 1 c.p.s. (in X and Y) were recorded on August 7, 1959, more than 500 miles apart at the Albert Head base station and at Ralston in the Canadian Prairies. Prof. Hugo Benioff informed us that they were also recorded photographically for all three fluctuating elements at his Isabella Station in Southern California. At Ralston they were recorded on magnetic tape and the amplitude of the vertical (Z) component was found to be inappreciable for this frequency. At Albert Head, the vertical detector system only was being used, but the signals were of large amplitude.

The interesting feature is the inappreciable vertical values at Ralston and the large values at Albert Head. We account for the small vertical component at Ralston as due to a uniform Earth conductivity field at least to the (unknown) 'skin depth' of 1 c.p.s. signals. This result was scarcely unexpected as the

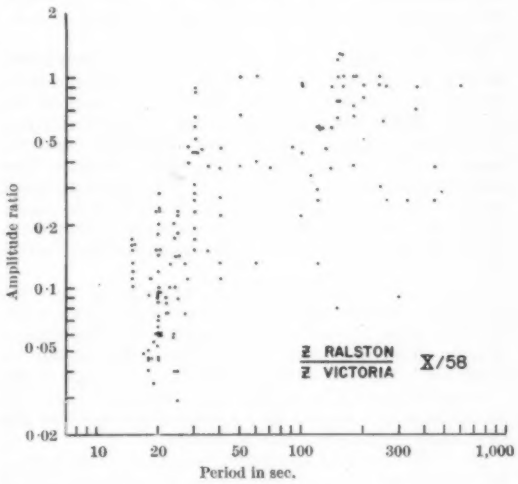

Fig. 1. Ratio of vertical force fluctuations at Ralston and Victoria, in October 1958

Fig. 2. Record of vertical force fluctuations at Victoria. This shows high- and low-frequency components in upper trace; high frequency (about 1 c.p.s.) only in lower trace

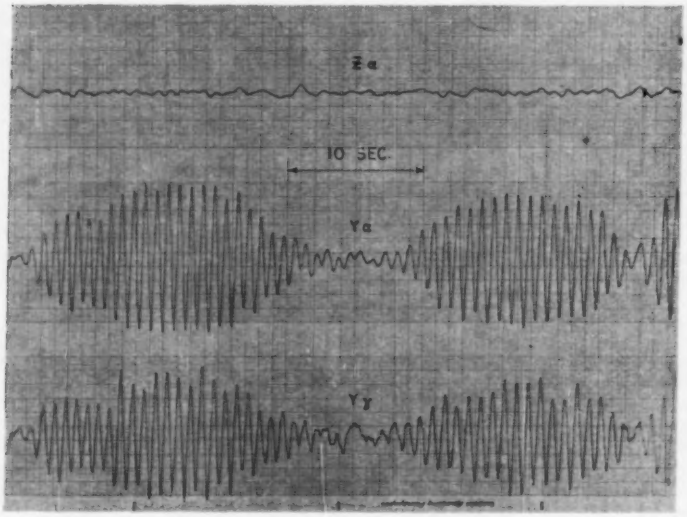

Fig. 3. Record of 1 c.p.s. signals at Ralston about midnight on August 7, 1959. Transferred from magnetic tape record

site was originally chosen as an area of probable geological uniformity. Here, the surface of the Pre-Cambrian rock is nearly horizontal, the thickness of the over-burden probably varying only from 6,000 ft. to 7,000 ft. in a distance of some 50 miles.

The large value of vertical force fluctuation at Albert Head is, of course, due to the situation of the detectors on the shore of the Strait of Juan de Fuca, where there is a large horizontal gradient of conductivity.

It should be added that the lower-frequency records at Ralston suggest that the geological conditions are uniform horizontally to considerably greater depths than the 'skin depth' for the 1 c.p.s. background signal. In conformity with this view, we have found a very close correlation between the background signals at two places at Ralston 5½ miles apart for the frequency-range of 0·02–0·1 c.p.s., using the digital computation technique employed by Prof. Walter Munk in his investigation of swell phenomena[2].

Over this short distance the correlation between the two stations has been found to be very high for the longer periods. However, at frequencies greater than 0·1 c.p.s. an unfavourable signal to noise ratio was encountered, partly as a result of the normally lower amplitudes and partly because of limitations due to the slow speed (0·15 i.p.s.) of the frequency modulation tape recorder.

The fact that one can find fluctuations of so high a frequency as 1 c.p.s. occurring simultaneously at places many hundreds of miles apart argues, we think, that these signals originate out beyond the ionosphere, or else that the initiating system is of dimensions large compared with 1,000 miles, or both. However, the cause of the intermittent occurrence (Fig. 2) at about 2-min. intervals seems unlikely to be a characteristic of the origin and we have not been able to assure ourselves that the envelope of the recorded amplitudes occurs simultaneously at two widely separated stations, a phenomenon which is usual with fluctuations of frequency between 0·03 and 0·1 c.p.s. The examination of numerous records of these lower frequencies gives some confidence that the times of the maxima and minima of the individual fluctuations are simultaneous within less than 1 sec.—at least on the average and judging only by the occasions when the records are so similar that individual fluctuations can be identified.

There remains the question of how far inland the effect of the sea can be traced for the different frequencies which compose the background. The importance of local topographical differences is one which can only be settled effectively by much detailed work. This may well permit the delimitation of areas of geological similarity within which the geomagnetic background is similar. Parkinson[3] discusses the recorded fluctuations of much longer period in Australia and considers the possibility that the differences between the records at these stations may be due to the highly conducting oceans. However this may be, there can be no doubt that distance from the sea is a factor of great importance for fluctuations of the frequency with which we are concerned. We also believe that comparison of the fluctuations at two well-separated stations, each situated in an area of geological uniformity and not too close to an auroral zone, would show a much better agreement in amplitudes and phases of the corresponding components (X, Y and Z) than we have yet observed except at the two contiguous stations at Ralston.

We are much indebted to Mr. A. M. Pennie, chief superintendent of the Defence Research Board Experimental Station at Ralston, and to his staff for help and assistance; to the Superintendent and staff of the Dominion Experimental Farm at Summerland for their kindness to our party during the experiments there; to Dr. Roger Revelle, director of Scripps Institution of Oceanography, and Prof. Walter Munk and his staff for help in dealing with data-records; and to the Royal Canadian Navy for the use of the Albert Head facilities for accommodation of our base station.

This work is part of the programme of the Pacific Naval Laboratory of the Defence Research Board of Canada. One of us (C. S. W.) is working under contract with the Defence Research Board.

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LETTERS TO THE EDITORS

PHYSICS

Reflectance of Coal Vitrains in the Visible and Ultra-Violet compared with that of Graphite

RECENT work[1] on the spectral variation of the reflectance of graphite has shown that there is a strong peak of reflectivity at about 2600 Å. This is associated with a marked absorption band at 2500 Å., which can be accounted for in terms of π-electron transitions. The well-known absorption of many cata-condensed hydrocarbons at about the same wave-length has been similarly explained. It was decided to extend the investigation to coal in the hope of throwing some light on the molecular constitution; the X-ray studies of Hirsch[2] are open to conflicting interpretations[3].

Light from a xenon arc was passed through a Hilger quartz monochromator and caused to fall as a parallel beam on the specimen at almost normal incidence. The reflected light was detected by a photomultiplier 'Mazda' 27 *M* 3 (similar to 'RCA' *IP*.28), the output of which was fed into a Muirhead a.c. amplifier tuned to 100 c./s., the arc modulation frequency. At each wave-length the intensity of the reflected light was compared with that reflected from a smoked magnesium oxide surface placed in the incident beam just before the specimen. The ratio of the two intensities, corrected for the slightly decreased reflectance of the magnesium oxide in the ultra-violet, gives the reflectivity of the specimen in arbitrary units. Absolute values may be obtained by replacing the magnesium oxide surface with a thin parallel-sided plate of fused silica; such values are not, however, given here because an unsuspected anomaly in the reflectance of certain samples of fused silica has been found below 2800 Å. and is still being studied.

The specimens, kindly provided and prepared by the British Coal Utilization Research Association, were all vitrinites cut parallel to the bedding plane and highly polished; details are given in Table 1.

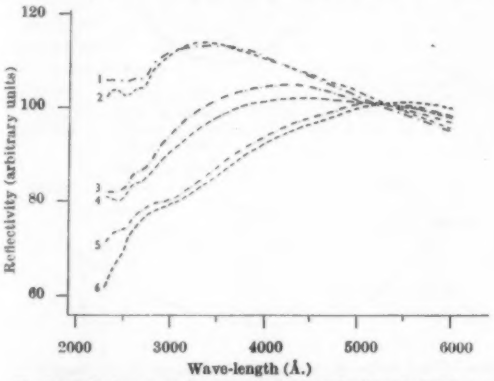

Fig. 1. Reflectance spectra of coal vitrains (identified in Table 1). Reflectivities are in arbitrary units referred in each case to 100 at 5400 Å.

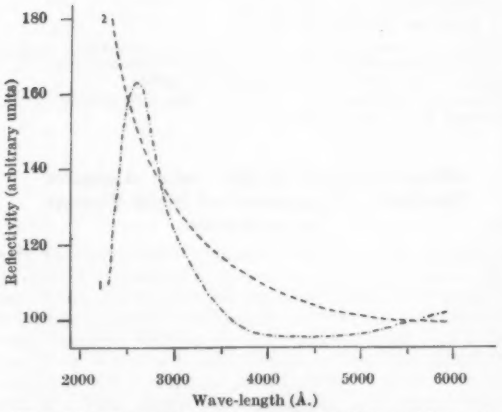

Fig. 2. Reflectance spectra of (1) graphite and (2) type II diamond. Reflectivities are in arbitrary units referred in each case to 100 at 5400 Å.

The results, adjusted for a reflectivity of 100 at 5400 Å., are shown in Fig. 1. Three distinct types of spectra are apparent, comprising respectively specimens 1 and 2, 3 and 4, 5 and 6. A detailed study is now being made of the relationship between the type of reflectance spectrum and carbon content. In due course a full report will be published elsewhere.

Fig. 2 shows the corresponding spectra of a highly compressed, polished sample of colloidal graphite and of a type II diamond. The very distinct graphite peak does not appear in the coal spectra, although a slight shoulder is detectable between 2700 Å. and 2600 Å. in some of the specimens. As it is generally the case that reflectance and absorption variations are closely related, this might indicate that none of the coals exhibits a significant absorption of aromatic type in the neighbourhood of 2600 Å. On the other hand, the difference in the spectra of the coals of high and low rank could be caused by the increased presence in the latter case of structures the absorption of which increases with decreasing wave-length.

Friedel and Queiser have examined[4] a Pittsburgh vitrain, containing 84 per cent carbon, by both reflexion and transmission. A slight shoulder was found at 2,650 Å., but the absence of any definite absorption band is reported.

Acknowledgment is made to the National Coal Board for a grant in aid of this investigation, to Dr.

Table 1

Specimen	Origin	Carbon (per cent)
1	Dinnington Colliery Barnsley Seam	85
2	Vivian Colliery Meadow Vein	88
3	Coegnant Colliery Gellideg Seam	91·4
4	Glyncorrwg Colliery 9-ft. Seam	92·5
5	Blaenhirwaun Colliery Stanllyd Seam	94
6	Aberpergwm Colliery 9-ft. Seam	93·1

S. P. F. Humphreys-Owen of Birkbeck College, University of London, for helpful discussions, and to the Principal of Hendon Technical College for permission to submit this communication for publication.

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Measurements of the Total Acoustic Radiation Impedance of Rigid Pistons in an Array

Measurements have recently been made of the radiation impedance of sound projectors working in arrays. The projectors used each consist of a 45° Z-cut ammonium dihydrogen phosphate crystal cemented to a flint-glass piston. The projectors resonate near 18 kc./s. and have a square face of side $a = 0{\cdot}55$ in., so that $ka = 1{\cdot}050 = 2\pi a/\lambda$, where k is the propagation constant of the fluid load, water, and λ is the sound wave-length in water. The equivalent circuit of this simple sound projector is known so that from a measurement of the electrical admittance of any projector near resonance it is possible to deduce both the radiation resistance and reactance at the piston diaphragm. For these measurements the projectors were driven in parallel at 3 volts, and the resonant frequencies of the projectors in air differed by less than 0·2 per cent.

It has been found that the admittance diagram of a projector is a circle, indicating a constant radiation impedance, only when that projector is part of a two-element or square four-element array, that is, configurations in which the sound field conditions are the same for each projector. Fig. 1 shows the admittance circle of one projector in a square four-element array where the distance between the centres of neighbouring projectors is only 0·58 in. $= 1{\cdot}06a = 0{\cdot}18\lambda$. In a square array containing any other number of projectors there is no unique value of projector impedance since this depends on the position of a projector in the array. For these arrays the measured admittance diagram is exceedingly complicated and Fig. 2 is a typical example of such a plot. This is the admittance diagram of one projector in a square 36-element array in which the distance between the centres of neighbouring projectors was 0·654 in. $= 1{\cdot}19a = 0{\cdot}20\lambda$. The off-resonance frequencies in these diagrams do lie on a circle showing that at these frequencies the radiation impedance is constant; but near the resonant frequency

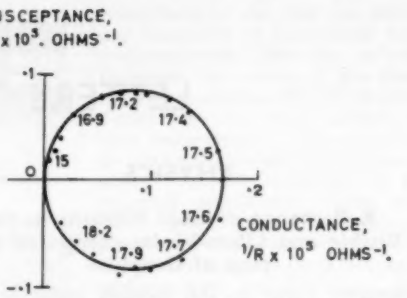

Fig. 1. Admittance circle of one projector in a square four-element array. Square diaphragms, $ka = 1{\cdot}050$ in water. Separation between centres of projectors is $1{\cdot}06a$. Frequency in kilocycles

the admittance increases suddenly and its real part, the conductance, may become negative. This is shown by the broken line in Fig. 2.

If an array has a frequency-modulated input this increase in admittance would cause sudden changes in both the diaphragm displacement and driving current at frequencies close to resonance.

It seems likely from a preliminary theoretical investigation that the phenomenon is due to large changes in the relative phase of the diaphragm velocities of neighbouring projectors near resonance. The changes occur because each projector is subjected to a different reactive loading, so that near the mean resonant frequency of the array the velocity vectors of each diaphragm would rotate at different angular velocities. To a first-order approximation the total radiation impedance on the ith projector in the array is given by:

$$Z_i = Z_{i1} \cdot \frac{v_1}{v_i} + \ldots Z_{ir} \cdot \frac{v_r}{v_i} + \ldots Z_{ii} + \ldots Z_{in} \cdot \frac{v_n}{v_i}$$

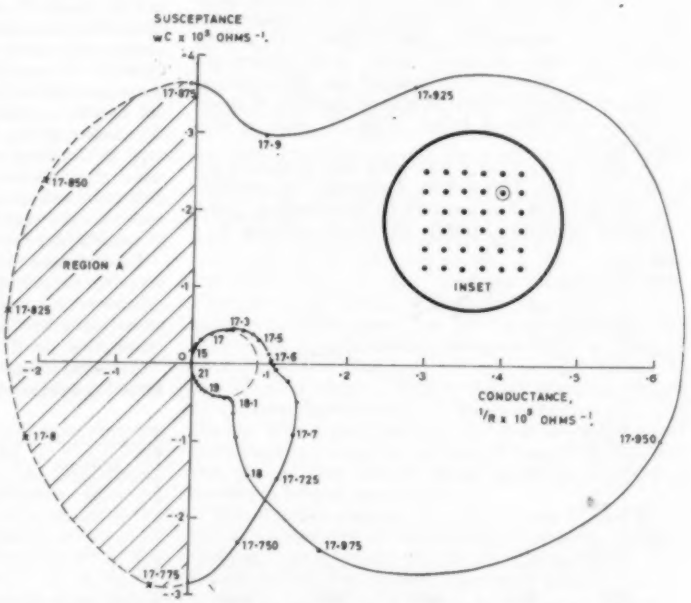

Fig. 2. Admittance diagram of one projector in a square 36-element array. Square diaphragms, $ka = 1{\cdot}050$ in water. Separation between centres of projectors is $1{\cdot}19a$. The position of the measured projector in the array is shown in the inset by a circle. In region A the susceptance values are known, but the negative conductance values are unknown. Frequency in kilocycles

where v_r, etc., are the complex diaphragm velocities, Z_{ii} is the self-radiation impedance and Z_{ir}, etc., are mutual radiation impedance coefficients. Now in close-packed arrays containing projectors which have diaphragms that are small in terms of a wavelength (say, $<\lambda/3$) the mutual radiation impedance terms $Z_{ir}.v_r/v_i$, etc., are dominant. It is believed then that the argument of these complex terms will vary rapidly with frequency near the mean resonant frequency of the array.

Detailed calculations are planned to test this suggestion.

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Ultrasonic Propagation Constants in Supercooled Salol

Discontinuities in various physical properties[1] of liquids in the supercooled region have been observed by several workers. The present work was undertaken to investigate whether such changes occur in ultrasonic velocity and absorption in the supercooled region of the liquid.

The liquid chosen for study was salol (phenyl salicylate), which was selected for the sake of convenience of its melting point. The velocity measurements were made with the help of a precision interferometer capable of giving values within 0·5 m. in a thousand. The observations were taken at 6 Mc./s. with the experimental arrangement[2] used here earlier.

The ultrasonic absorption was measured by the disappearance of the central order in the diffraction pattern, a method[3] developed earlier here. The investigations were carried out at 7·15 Mc./s. Arrangements were made for keeping the different temperatures constant within 0·1° C. by a thermostat controlled by an electric relay. The range of temperature studied was 35–58° C., passing through the melting point at 42° C. The results obtained, which were consistent in half a dozen experiments, are given in Table 1 and Fig. 1.

Results obtained in salol for velocity and absorption show a discontinuity near the melting point, the temperature coefficient of absorption being different on either side of the melting point. The slope of the temperature – absorption curve in the normal region is comparatively greater than that in the supercooled region, whereas there is no appreciable difference observed in the temperature coefficient of velocity on either side of the melting point. It is seen that the abrupt change in absorption coefficient observed near the melting point is definitely outside the range of experimental error.

Table 1

Temperature (° C.)	Velocity (m./sec.)	Absorption (10^{17} cm.$^{-1}$ sec.^{2})
35·0	1,447·0	680
37·0	1,440·5	700
39·0	1,434·0	715
41·0	1,428·0	730
42·0	Melting point	Melting point
43·0	1,418·0	820
46·0	1,409·0	910
49·0	1,399·5	1,000
52·0	1,390·0	1,090
55·0	1,380·5	1,180
58·0	1,371·0	1,270

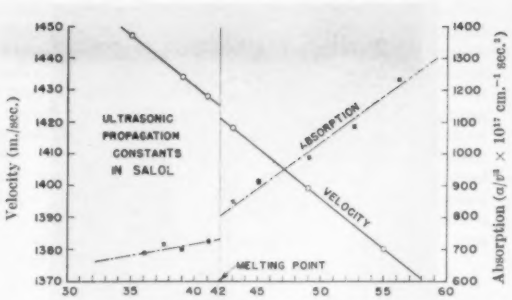

Fig. 1

It has been observed by Dodd[4] and his co-workers that the temperature coefficient of density shows no detectable change as the liquids are supercooled. This suggests that the adiabatic compressibility must show a sudden change at the melting point. If this phenomenon be due to a sudden change in adiabatic compressibility, a corresponding discontinuity may be expected in ultrasonic velocity and absorption coefficient near the melting point of the liquid. This type of discontinuity is confirmed by the present investigation.

Further work on the problem is in progress the details of which will appear elsewhere.

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Feb. 5.

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An Inexpensive Castle and Sample Holder for Radioactive Counting

The use of radioactive tracers is becoming much more extensive thanks to the training programmes in the more technologically advanced countries which offer courses to students from other regions of the world. The high initial cost of much equipment used to detect radioactivity is often the explanation for the time-lag in using the powerful tracer technique to solve important local problems in agriculture and medicine.

One means of reducing the cost is shown in the accompanying photographs. There is no necessity to buy expensive lead or aluminium castles to support the samples beneath the Geiger tube. The wooden holder (Fig. 1) for the Geiger–Müller tube is a solid block through which a hole was drilled slightly larger than the diameter of the Geiger–Müller tubes likely to be used. This block was then slotted to allow the butterfly clamps to be tightened enough to hold the tube in place.

The solid block was surrounded by wood sheets on four sides. The bottom serves not only as a brace but

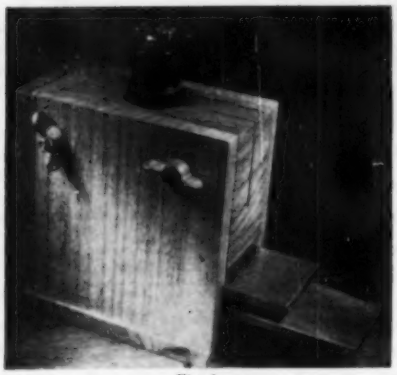

Fig. 1

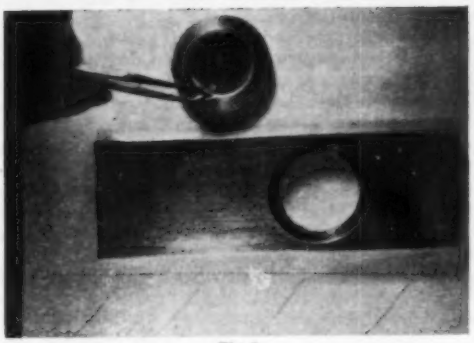

Fig. 2

also as a uniform back scatterer if the castle is moved from one table to another. The rear serves as a stop so that all the samples are uniformly aligned beneath the Geiger–Müller tube. The sides are grooved to carry the sample holders. The number of grooves and their distance apart can be suited to the user.

The sample supports (Fig. 2) were made on a metal lathe using stock steel ¼ in. thick and 2½ in. wide. A shelf was left at the bottom just large enough to support the steel ring which fits inside. This ring carries the sample pans with the radioactive material. The ring has a groove to make the handling of the pans easier. The convenience of the ring, which was made from bar steel, lies not only in the fact that it can be made to fit any size sample pan, but also it is excellent when a non back-scattering support is required. Fig. 3 shows a piece of thin 'Pliofilm' stuck between the ring and the support. The bottom photograph shows how back scatterers can easily be held in place.

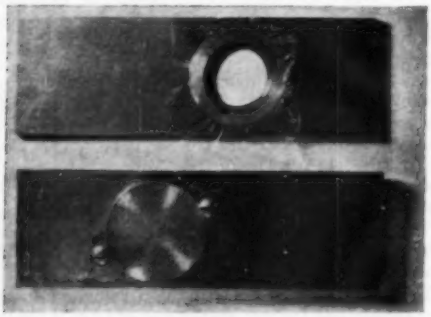

Fig. 3

The geometry has been reproducible with no difficulty. The wooden castle and two steel holders were made for about 5·00 American dollars.

The background using a 900-volt Geiger–Müller tube with a mica window of thickness 1·4 mgm./cm.² is about 70 counts/min. at an altitude of 600 m.

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Time-lags in the Intrinsic Electrical Breakdown of Glass

When measuring the electrical breakdown strength of lead glass sheets with 1-cm.-diam. sphere electrodes subjected to 0·1/1,500 μsec. voltage pulses it was found that the secondary effects, usually present when transformer oil or similar immersion media are used, could be eliminated by using low-conductivity water as the immersion medium. Under these conditions when the edge effects were suppressed it was observed that the breakdown took place after a time delay in the range of 10^{-7}–10^{-4} sec. The distribution of these time delays was found to be random. The results fit the relationship:

$$n/n_0 = \exp(-t/\tau)$$

where n is the number of observed time lags within any time t; n_0 is the total number of time lags observed; τ is a constant.

These results are comparable with the findings of Cooper and Grossart[1] on potassium chloride crystals, polythene and polystyrene and the experiments done by Kawamura[2–4] and his collaborators on mica.

The intrinsic electric strength of lead glass under low-conductivity water was found to be $11{\cdot}4 \pm 1{\cdot}1 \times 10^6$ V./cm. This is considerably higher than 6×10^6 V./cm. reported by Austen and Whitehead[5]. This work will be fully reported elsewhere.

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METALLURGY

Piling-up of Dislocations beneath a Surface Film

The concept of a surface film obstructing the egress of dislocations from a crystal and thereby causing them to pile-up beneath the film has proved useful in explaining the results of several metallurgical experiments[1,2]. However, direct evidence for such a phenomenon has been lacking. During an investigation to study some aspects of the effects of surface coatings—oxides, metallic and surface-active films,

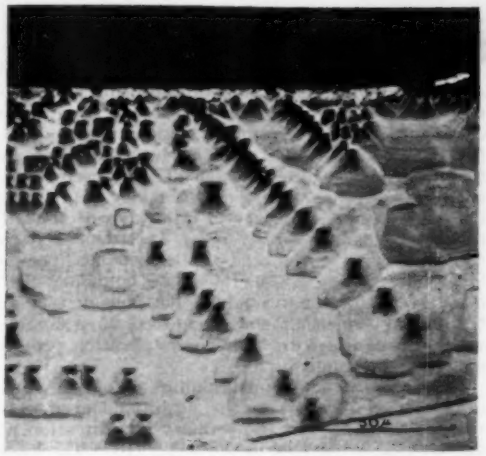

Fig. 1

etc.—on the mechanical behaviour of materials, several examples of piled-up groups of dislocations have been observed. Fig. 1 shows a pile-up of edge dislocations beneath a surface coating in lithium fluoride. The coating was formed by reaction of the crystal with pure magnesium oxide powder at 600° C.; the dislocations were revealed by the techniques developed by Gilman and Johnston[3].

Examples of piled-up groups of both edge and screw dislocations at sub-boundaries, the piling up of edge dislocations on one slip system against a dense band of edge dislocations on another, and the subsequent production of a Stroh-type[4] crack have also been observed in lithium fluoride.

A more detailed account of the investigation will be published at a later date.

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A Technique for Sampling Oxide Layers and Brittle Materials for Microdiffraction

In the course of investigations on corrosion in electrical generating plant, metallographic examination of polished sections of various corrosion products revealed a layer structure and the morphology of the different layers suggested that each consisted of one or more distinct phases. It was desired to use X-ray or electron-diffraction techniques to identify the constituents of the different layers, some of which were only a few microns thick.

Attempts to sample the materials from different layers by means of suitable tools held in a micromanipulator were unsuccessful and some of the layers were too thin for the application of micro-drilling techniques[1,2]. Experiments using a carbon extraction replica technique were also unsuccessful, as might be expected from the homogeneity of the layers, since such extraction techniques can only be expected to release relatively small particles of one phase from a matrix consisting of another phase. Etching techniques are also undesirable where both phases may be chemically similar, owing to the possibility of reaction between the etchant and the extracted phase. The possibility of loosening material by means of a diamond point and incorporating this into a replica for examination by electron diffraction was therefore explored.

The first experiments were made by drawing a glass-cutting diamond lightly along a layer in a polished section of a boiler tube corrosion product and making a 'Formvar' replica of the surface. The selected area 'Formvar'–carbon replica method described by Bradley[3] was used to arrange the groove made by the diamond across the middle of an electron microscope grid. Fig. 1 is a low-magnification electron micrograph (×200) of a replica made in this manner showing that the debris produced by the diamond was incorporated in the replica and that this is of a suitable particle size for electron-diffraction examination.

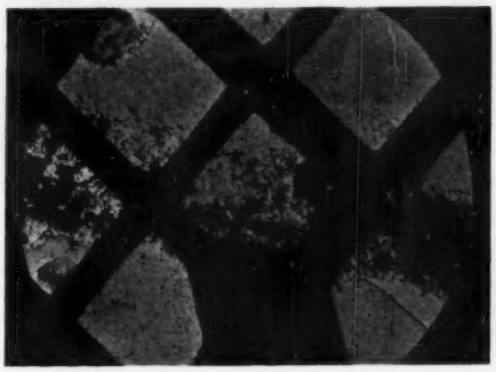

Fig. 1. Replica incorporating debris produced by diamond point. × 200

To produce reliable results by this technique, it is necessary to place the groove entirely within any desired region of the specimen, to avoid the possibility of debris being drawn from one region into another. A diamond engraving tool with a pyramidal angle of 90° was therefore mounted in a microhardness testing machine, which had a compound table fitted with micrometer screws for traversing, so that it could be brought into contact with the specimen at a point corresponding to the intersection of the cross-wires in the viewing microscope. A groove was then produced by traversing the specimen under the diamond point, which was loaded to between 1 and 10 gm. Fig. 2 is an optical micrograph (×90) showing two grooves, each about 5μ wide, produced in this manner in the inner layer of a corrosion product; the grooves appear double owing to damage caused to the diamond during preliminary experiments. A 'Formvar'–carbon replica incorporating material from one of these grooves was examined in an electron microscope, and Fig. 3 is a resultant micrograph (×8,800), showing the region examined by selected area electron diffraction. The pattern

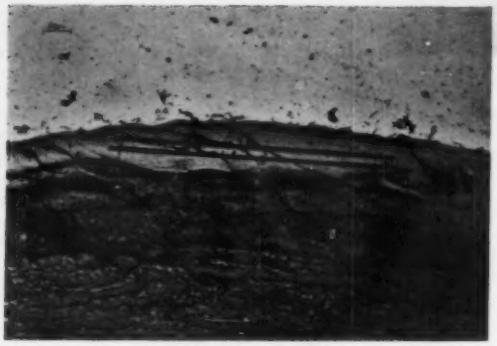

Fig. 2. Grooves located in layer of corrosion product. × 90

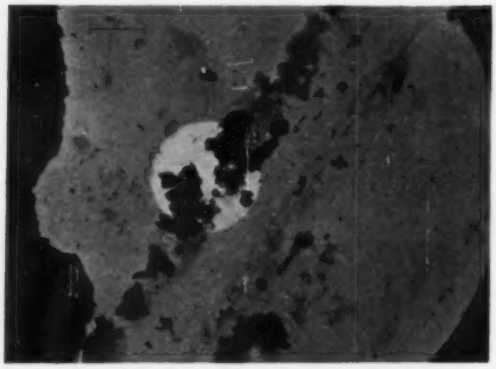

Fig. 3. Replica from part of a groove, showing region examined by selected area diffraction. × 8,800

from this region identified this material as γ-ferric oxide, whereas the material forming the outer layer in this sample was identified by a similar technique as magnetite.

The technique has been tested successfully on specimens of other brittle materials and it has been found that a load of 1 gm. normally produces sufficient debris for examination without risk of damaging the diamond. Materials that can be deformed plastically do not yield loose debris and the diamond groove technique is therefore unsuitable for these. However, it appears to offer a useful extension to the normal extraction replica techniques, which are often difficult to apply to brittle materials.

We are grateful to Mr. A. W. Agar, head of Aeon Laboratories, for advice, and one of us (A. M. A.) wishes to thank Dr. J. S. Forrest, director of the Central Electricity Research Laboratories, for permission to publish this communication.

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First Stage of Work Hardening in Aluminium Single Crystals

Previous work[1] has indicated that the only metallographic features appearing during stage I of the deformation of single crystals of face-centred cubic metals (the 'easy glide' region) is slip on the (111) plane of maximum resolved shear stress (laminar flow[2]). The transition to stage II has been related to the appearance of slip on secondary (111) planes (turbulent flow[2]). However, it is known[1] that the rate of hardening during stage I is dependent on the orientation of the axis of the specimen, particularly with respect to the [100]–[111] boundary of the basic stereographic triangle. This has led to the theoretical conclusion[3] that some secondary slip must occur during stage I. This communication provides experimental confirmation.

Crystals of 99·99 per cent aluminium of rectangular cross-section (grown by the strain-anneal method) were selected so that the primary slip direction was approximately parallel to the wider face. The height of the primary slip bands was thus small on this surface, thereby increasing the chance of detecting any secondary slip. After electro-polishing and etching, the crystals were strained successive amounts in a Polanyi machine with intervening metallographic examination by a phase-contrast microscope and the Schulz microfocus Laue technique[4].

The rates of hardening observed during stage I varied from 1·0 to 4·5 kgm./mm.²/unit shear strain, depending on orientation. In all crystals in which this rate was greater than about 1·6 kgm./mm.²/unit strain, and occasionally in those showing a lower rate, other octahedral systems were observed during stage I.

Table 1 gives the critical resolved shear stresses ($\sigma_{crit.}$), the rates of hardening ($d\sigma/d\varepsilon$) in stages I and II, and the lengths of stage I (ε_I) for the two crystals the orientations of which are specified by Fig. 1. ψ is the angle between a slip direction and the wider surface of the crystal. $\sin\psi$ is thus a measure of the component of slip normal to the surface.

Table 1

Crystal	$\sin\psi_P$	$\sin\psi_{CR}$	$\sin\psi_{CO}$	$\sigma_{crit.}$ in gm./mm.²	ε_I (per cent)	$(d\sigma/d\varepsilon)_I$ (kgm./mm.²)	$(d\sigma/d\varepsilon)_{II}$ (kgm./mm.²)
A	0·19	0·79	0·12	52	1·8	2·0	6·8
B	0·09	0·59	0·34	58	0·5	4·4	10·6

In crystal *A*, uniformly distributed slip bands on the critical system (relative resolved shear stress 86 per cent of that on the primary system) were already visible at 0·9 per cent shear strain (Fig. 2). The increasing height of the primary slip bands tended to mask the critical slip bands at the later inspections at 1·5 and 1·9 per cent shear strain. It has been concluded from examinations of this and other crystals of neighbouring orientation that the disparity in the relative rates of growth of the two sets of slip bands is due to the stimulation of slip in another direction in the primary plane (relative resolved shear stress 57 per cent). No additional slip systems were visible at 1·5 or 1·9 per cent strain, but traces of the conjugate system (relative resolved shear stress 73 per cent) were detected at 2·3 per cent shear strain (in stage II). Conjugate slip was most prominent within bands of secondary slip (Fig. 3)

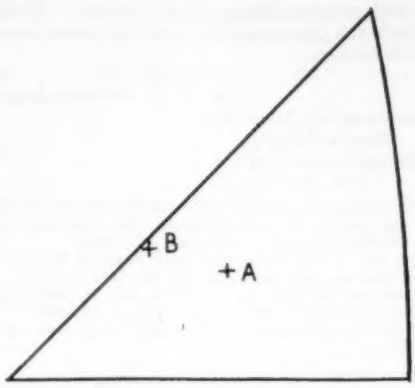

Fig. 1. Orientations of the axes of the crystals

but was just detectable all over the surface. The mis-orientations due to these bands of secondary slip were, however, already detectable by the X-ray method at the transition to stage II (1·9 per cent shear strain), so, taking the geometrical arrangement into account (Table 1), it is concluded that conjugate slip had operated at least from the start of stage II.

Stage I can be distinguished from stage II in crystals oriented near the [100]–[111] boundary such as *B* (Fig. 1). In this crystal, in which the relative resolved shear stress and the shear normal to the surface for the conjugate system were much greater than for crystal *A* (Table 1), uniformly distributed conjugate slip along with slip on the critical plane (relative resolved shear stress 87 per cent) and the additional primary system (relative resolved shear stress 44 per cent) were observed in stage I. The rate of hardening was thus very high and the extent of stage I very short. The transition to stage II again coincided with the detection of many bands of secondary slip containing slip bands on the conjugate plane.

These experiments suggest that the rate of hardening in stage I is determined by the amount and type of uniformly distributed secondary slip. The end of stage I seems to involve activity on several systems, and occurs rapidly with even only minor amounts of slip on the conjugate system.

These results may be interpreted theoretically in terms of the probability of formation of Lomer–Cottrell sessile dislocations in two directions in the primary plane by interactions between the critical system and the additional primary system, and between the primary and conjugate systems. It is not clear whether bands of secondary slip which always appear near the transition to stage II are relevant to hardening in themselves or whether they are only a method of accommodation of lattice rotation made possible by the early stimulation of secondary slip.

A full account of these experiments will be published elsewhere. I am indebted to the Chief Scientist, Department of Supply, Australia, for permission to publish this communication.

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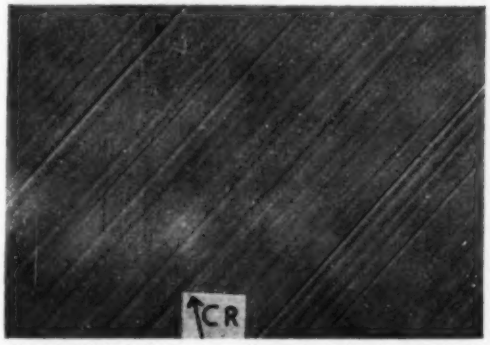

Fig. 2. Critical slip bands on the surface almost screw to the primary slip bands, 0·9 per cent shear strain. (× 100 negative phase contrast)

Fig. 3. Identical area to that of Fig. 2 at 2·3 per cent shear strain. Conjugate slip now visible. (× 100 negative phase contrast)

CHEMISTRY

Electron Transfer Reactions involving *s*-Trinitrobenzene

It has been claimed[1,2] that the reaction of *s*-trinitrobenzene with certain amines involves a proton transfer mechanism. Evidence given in support of this claim was not very satisfactory, and in fact it has been clearly shown by exchange measurements[3,4] on similar systems that no proton transfers occur. Other evidence is either conflicting, as is the case with certain electrolysis measurements[5,6], or irrelevant, having been obtained under very different experimental conditions[7].

We have recently shown[3] that *s*-trinitrobenzene forms ionic complexes with aliphatic amines and pyridine, in contrast to the non-ionic complexes which it forms with aromatic amines or hydrocarbons. Such complex formation is consistent with the following scheme :

$$C_6H_3(NO_2)_3 + R_3N \rightleftharpoons C_6H_3(NO_2^-)_3\overset{+}{N}R_3 \rightleftharpoons C_6H_3(NO_2)_3^{\bullet-} + R_3N^+$$

To obtain further evidence for this mechanism we have made electron spin resonance measurements on mixtures of *s*-trinitrobenzene with various amines,

either with the amine as solvent, or as concentrated solutions in acetone; we have also used the solid complexes where these could be prepared.

Mixtures of *s*-trinitrobenzene (*c.* 0·05 gm.) with the amines diethylamine and piperidine (*c.* 0·15 gm.) produced a weak spectrum after standing for about an hour, although no signals were detected initially. Similarly, equal volumes of a 1·5 molar solution of *s*-trinitrobenzene in acetone with either of these amines produced a spectrum the intensity of which gradually increased with time until a maximum was reached, after which it decreased.

A typical derivative curve of absorption spectrum is shown in Fig. 1. The g value is 2·00$_5$ and the line-width between points of extreme slope is 25 gauss. The curve corresponds to a three-peak absorption spectrum.

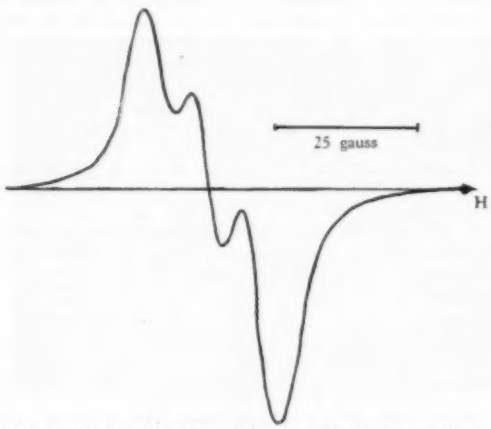

Fig. 1. Typical derivative of the absorption spectrum for a mixture of *s*-trinitrobenzene and diethylamine in acetone at 9,300 Mc./s.

The paramagnetic resonance spectrum in mixtures of *s*-trinitrobenzene with the aliphatic amines provides further strong evidence for an electron transfer producing the odd-electron ion $C_6H_3(NO_2)_3^-$; a proton transfer would produce the ion $C_6H_4(NO_2)_3^-$, which should show no signal. Furthermore, the decrease with time of the signal intensity in acetone solutions is in accordance with our earlier measurements and indicates that the product of the secondary reaction which occurs in these solutions[3] is not paramagnetic. The signals obtained from mixtures in which no acetone is present are presumably due to the ionic complex $C_6H_3(NO_2)_3^-.NR_2^+$, since we would not expect appreciable ionization in the amine solvents.

The g value and line-width of the absorption spectrum correspond closely to those reported for mixtures of *s*-trinitrobenzene with sodium in 1,2-dimethoxyethane[8]. In this case, however, eight peaks were observed, whereas we have observed only three. The solutions in these preliminary measurements are extremely concentrated and it is probable that more hyperfine structure would be observed in dilute solutions. Unfortunately, these dilute solutions lie outside the range of detection of our present apparatus, but we hope to obtain further measurements with an improved amplification system.

The results are also consistent with our view that complexes of *s*-trinitrobenzene with aromatic donors are not appreciably ionized in solution.

We wish to acknowledge the help of Mr. C. Jackson, who made the paramagnetic resonance measurements on our solutions.

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Radioassay by Gas Chromatography of Compounds labelled with Carbon-14

An efficient device for trapping materials from the effluent of a gas chromatography column has been developed which facilitates collection of components labelled with carbon-14 in close proximity to an efficient scintillator. Collection of components from aliquots of carrier gas into separate traps for subsequent counting affords a sensitivity of carbon-14 assay limited only by the high sensitivity of liquid scintillation counting technique. Continuous scintillation counting of a single trap through which the column effluent flows permits the high resolution and convenience afforded by simultaneously recording component elution and radioactivity.

Short sections of gas chromatography column placed at the exit port of an analytical column but kept at a lower temperature were found to retain the effluent materials quantitatively. Similar sections of glass tubing packed with anthracene crystals, which are coated with liquid stationary phase in the same manner that 'Celite' is coated with liquid phase to prepare an analytical column, were found to be similarly retentive. That anthracene crystals are efficient scintillators when placed in proximity to materials containing carbon-14 has been demonstrated by Steinberg[1], who reported the use of anthracene crystals for carbon-14 assay of amino-acids in aqueous solution. The carbon-14 activity of methyl esters of fatty acids trapped from a column effluent on coated anthracene has been assayed without further manipulation by substituting the trapping column for the sample of liquid scintillator solution in a liquid scintillation spectrometer.

When counted with sufficient high voltage to amplify all the spectrum above base-line, carbon-14 is counted in anthracene with almost the same efficiency as in toluene–*PPO*, with similar background-rates. The counting-rate measured in the coated anthracene was found to be linear with respect to the quantity of ester labelled with carbon-14 trapped from the chromatography column (Fig. 1). The rates corresponded closely with the rates obtained by pipetting the same amounts directly on trapping columns, which, in turn, did not differ appreciably from the rates obtained by pipetting the same amounts into a solution of *PPO*–toluene (6 mgm. *PPO* per ml. of toluene). Counting was performed in a Packard 'Tri-Carb' liquid scintillation spectrometer, using settings as follows: photo-tubes, 1,450 V.; window 10 V. to infinity.

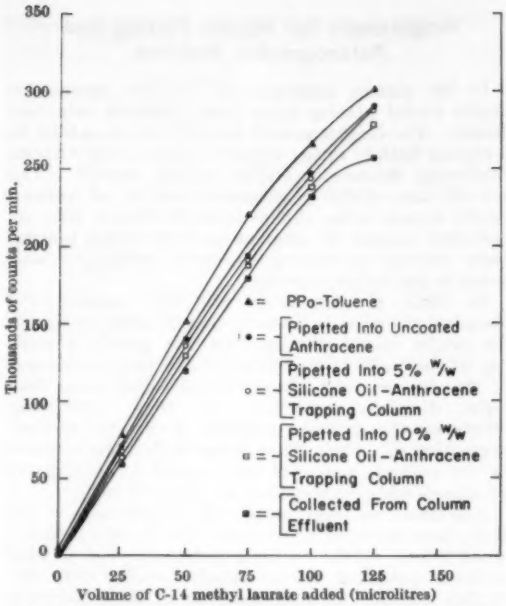
Thousands of counts per min.
Volume of C-14 methyl laurate added (microlitres)
▲ = PPo-Toluene
Pipetted Into Uncoated Anthracene
Pipetted Into 5% W/w Silicone Oil-Anthracene Trapping Column
Pipetted Into 10% W/w Silicone Oil-Anthracene Trapping Column
Collected From Column Effluent

Fig. 1. Counting response of trapping columns to graded amounts of ^{14}C-methyl laurate

The length of trapping column necessary and the amount for liquid phase were determined by measuring the distribution of radioactivity along 12-in. trapping columns of ¼ in. internal diameter. Methyl laurate labelled with carbon-14 was led from a column effluent (200° C.) into trapping columns at room temperature, following which the trapping columns were purged with hot effluent carrier gas for 1 hr. (Table 1). Collection of most of the radioactivity in the first inch of the coated anthracene column compared with more even distribution of the radioactivity on the uncoated anthracene suggested the use of trapping columns 2 in. long containing coated anthracene in subsequent experiments.

The stationary phase in the trapping column should retain the substances eluted from the analysis column in the shortest possible length, while interfering as little as possible with the scintillation process. The interference caused by a number of stationary phases is shown in Tables 2 and 3. Silicone oil ('Dow Corning-550') coated 5 per cent weight/weight on anthracene was selected for its low interference and high retentivity for fatty acid esters.

A continuous record of carbon-14 radioactivity emerging from the column was obtained using the same trapping device. A trapping column 2 in. long and ¼ in. internal diameter was fixed in position between the photo tubes of the scintillation counter. Effluent gas from the analytical column was conducted to this trapping column through a tube of 1/16 in. internal diameter maintained at 225° C. When the output of a counting-rate meter monitoring the total counting-rate in the trapping column is plotted on a strip chart, a record is obtained in which stepwise increments in counting-rates indicate the time of elution and the quantity of radioactivity contained in the components eluted. The sensitivity of continuous radioassay by this procedure is limited only by the statistical nature of the emitted radiation and the time between elution of components available for counting.

Although we have worked at the solution of the specific problem of the radioassay of fatty acid esters, the technique is clearly of more general applicability.

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Table 1. DISTRIBUTION OF RADIOACTIVITY ALONG TRAPPING COLUMN 12 IN. LONG FOLLOWING PURGING FOR 1 HR. WITH COLUMN EFFLUENT (COUNTS/MIN.)*

Segments	Uncoated anthracene	Anthracene coated with silicone oil 5 per cent w/w	Anthracene coated with silicone oil 10 per cent w/w
1	12,350	126,128	123,575
2	10,580	631	585
3	42,914	178	152
4	11,596	195	238
5	168	119	105
6	156	90	62
7–8	112	98	112
9–10	93	110	98
11–12	98	98	100

* Figures above include background counting-rate of 100 counts/min.

Table 2. EFFECT OF LIQUID PHASES ON COUNTING OF CARBON-14 IN *PPO*-TOLUENE LIQUID SCINTILLATION SYSTEM

Liquid phases	Percentage of total counts compared to *PPO*-toluene 5 per cent w/w	10 per cent w/w
1. Polyethylene glycol ('Carbowax 400', Union Carbide)	97	97
2. Succinate diethylene glycol	97	93
3. Silicone oil ('Dow Corning 550 Fluid')	96	93·5
4. Silicone grease ('Versilube *G*-300', General Electric)	91·9	81·1
5. 'Reoplex 400' (Geigy)	86	79·9
6. Dioctylphthalate ('Octoil')	64	42
4. 'Apiezon L'	43	28

Table 3. RELATIVE EFFECTS OF 5 PER CENT W/W LIQUID-PHASE COATINGS ON COUNTING OF CARBON-14 IN ANTHRACENE SYSTEM

Liquid phases	Percentage of total counts compared to *PPO*-toluene
1. *PPO*-Toluene	100
2. Uncoated anthracene	93
3. Dioctylphthalate	92
4. Methyl stearate	90
5. Silicone oil ('Dow Corning 550 Fluid')	89
6. 'Reoplex 400'	83·5
7. Silicone grease ('Versilube *G*-300')	79
8. Adipate diethylene glycol polyester	71
9. Polyethylene glycol	67·5
10. Succinate diethylene glycol polyester	66
11. 'Apiezon L'	65
12. Tetra hydroxyethylethylene glycol	47·5

Ionization of *n*-Paraffin Molecules

THE ionization potentials of the members of the *n*-paraffin series of hydrocarbons have been calculated on the assumption that the electron which is removed comes from a symmetrical molecular orbital[1]. Coggeshall[2] has used these molecular orbitals to investigate the density of positive charge in the ion and has shown that there is no relation between this density and the mode of fragmentation of the ion. Field and Franklin[3] have suggested that there might be some connexion.

It is possible that the electron which is removed on ionization comes from an anti-symmetrical molecular orbital and this has been investigated also by Hall[4]. He shows that this assumption is in as good agreement with the experimental data as the previous one. It is of interest, therefore, to see if the break-up of the ion can be related to the charge distribution predicted when the highest anti-symmetrical molecular orbital is singly occupied.

When the highest symmetrical molecular orbital is singly occupied the resultant charge distribution can be expressed as a distribution over the C–C bonds, since this symmetrical molecular orbital is obtained predominantly in terms of equivalent C–C bond orbitals. However, an anti-symmetrical molecular orbital is obtained in terms of equivalent orbitals centred on the CH_2 groups. Thus, although the resultant charge distribution in the ion is similar to that obtained by Coggeshall (it is greatest at the centre and least at the ends), this charge distribution is not associated directly with the C–C bonds.

For an n-paraffin molecule with n carbon atoms (C_nH_{2n+2}) the coefficients at CH_2 group i ($i=1,2,\ldots n$) of the anti-symmetrical molecular orbitals are:

$$\sqrt{\left(\frac{2}{n+1}\right)} \sin\left(\frac{ir\pi}{n+1}\right)$$

where $r = 1,2,\ldots n$. The n values of r give n anti-symmetrical molecular orbitals and $r = n$ corresponds to the highest orbital, which is the one from which the electron is removed. For this molecular orbital the coefficients are[5]:

$$(-1)^i \sqrt{\left(\frac{2}{n+1}\right)} \sin\left(\frac{i\pi}{n+1}\right) \qquad (1)$$

and thus the charge distribution is given by:

$$\left(\frac{2}{n+1}\right) \sin^2\left(\frac{i\pi}{n+1}\right),\ i=1,2,\ldots n$$

Since the coefficients (1) alternate in sign the product of two adjacent coefficients is always negative and an electron in the highest anti-symmetrical molecular orbit contributes a negative amount to the bonding matrix: that orbital is anti-bonding. On ionization all bond orders are increased and the centre bond orders are increased by the greatest amount. Thus if there were any relation between the bond order and the probability of bond rupture this result would predict that the end C–C bond in the ion would break most frequently. This is not in accord with the experimental data, which show that the break-up occurs most often at the C–C bond which is third from the end[3].

The existing methods for calculating ionization potentials have sound theoretical bases, but the properties (charge and bonding matrices) of the molecular orbits for the hydrocarbon cannot be used directly to predict the behaviour of the ion. Since the break-up of the ion seems to depend on the thermochemical stability of the resultant fragments, the problem will require a detailed investigation of the structure of the ion.

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Brighteners for Nickel Plating and Polarographic Maxima

In the plating industry, the addition agents for bright nickel plating have been classified into two classes. The brighteners of the first can be added to a plating bath in rather higher concentration without conferring deleterious effects on the deposit. The second class of the brighteners, used in all modern bright nickel baths, differs from the first in that its members cannot be used alone in a nickel plating bath without producing excessive brittleness and stress in the nickel deposits.

In 1953, H. Leidheiser and his co-workers[1,2] classified the various kinds of organic addition agents for bright nickel plating into five groups according to their effects on the grain size and orientation of the deposit, and afterwards classified them into eight divisions according to their increasing effects on the cathode potential during the electrodeposition of nickel. They inferred that the increase in the cathode potential was caused by adsorption of organic addition agents on the cathode surface.

According to their result, the brighteners of the first class belonged to division 1 or 2, which had a small effect on the cathode potential, and yielded cathode potential curves which levelled off with further increase in concentration of these addition agents; and on the other hand, the brighteners of the second class belonged to other divisions which had a moderate to great effect on the cathode potential.

In 1954, an interesting relationship between the corrosion inhibiting effect of the substance and the effectiveness of suppressing the polarographic maximum was reported by H. C. Gatos[3].

In the present communication, an attempt is described to relate the classification of the brighteners according to their increasing effects on the cathode potential to the effectiveness of suppressing the polarographic maximum. The effectiveness of various brighteners in suppressing polarographic maxima is expressed as their maximum suppression points. This is defined as the maximum concentration of the brightener which is necessary to make the maximum current, i_m, equal to the limiting (diffusion) current, i_l; it was determined graphically by plotting i_m/i_l against the logarithm of the concentration of the brightener.

The addition agents used were selected from among the division 1, 2, 5, 6 and 7 described in ref. 2, with the exception of naphthalene-1-sulphonate and naphthalene-1,3,5-trisulphonate, which were usually used as brighteners of the first class in the plating industry.

The maximum suppression point of various organic addition agents for copper (1·00 mM) and nickel (2·64 mM) maxima in 0·1 M potassium nitrate solution are shown in Table 1, where the divisions of the brighteners according to their increasing effects on the cathode potential and the lowest concentration at which brightening was noted are also shown.

As seen in Table 1, the brighteners of Nos. 1–7, which were usually used as the first class of the brighteners and belonged to division 1 or 2, were observed to be remarkably effective for the copper maximum which appeared at the positive side of the electrocapillary zero, and were scarcely or not effective at all for the nickel maximum which appeared at the negative side of the electrocapillary zero. On the contrary, the brighteners of Nos. 8–12

Table 1. MAXIMUM SUPPRESSION POINT OF VARIOUS ORGANIC BRIGHTENERS FOR COPPER AND NICKEL MAXIMA

No.	Organic brighteners	Classification according to their increasing effects on the cathode potential (according to ref. 2)	Lowest concentration at which brightening was noted (i_m/i_l)	Maximum suppression point (m/l)	
				Copper	Nickel
1	Benzene sulphonate (Na salt)	Division 1	0·02	$1·67 \times 10^{-3}$	8×10^{-3}†
2	Naphthalene-1,3,6-trisulphonate (Na salt)	1	0·0006	$2·29 \times 10^{-4}$	5×10^{-3}†
3	Naphthalene-1,5-disulphonate (Na salt)	2	0·0002	$2·71 \times 10^{-4}$	5×10^{-3}†
4	Naphthalene-1-sulphonate (Na salt)*	—	—	$7·90 \times 10^{-4}$	10^{-3}‡
5	Saccharin	2	0·0006	$1·28 \times 10^{-5}$	10^{-3}‡
6	*p*-Toluene sulphonamide	2	0·0015	$1·29 \times 10^{-3}$	$7·5 \times 10^{-4}$†
7	Naphthalene-1,3,5-trisulphonate (Na salt)*	—	—	$2·54 \times 10^{-4}$	$7·5 \times 10^{-4}$†
8	Quinoline	5	0·003	$4·82 \times 10^{-4}$	*c.* $1·5 \times 10^{-3}$
9	Quinaldine	6	0·001	$4·63 \times 10^{-4}$	*c.* 10^{-5}
10	*Isoquinoline*	7	0·001	$1·84 \times 10^{-4}$	*c.* $2·5 \times 10^{-5}$
11	Quinoline ethiodide	7	0·001	$1·74 \times 10^{-4}$	$2·57 \times 10^{-4}$
12	Quinaldine ethiodide	7	0·0005	$1·07 \times 10^{-4}$	$1·05 \times 10^{-4}$

* These addition agents have been used as the brighteners of the first class in the plating industry and perhaps may be classified in division 1 or 2 according to their increasing effects on the cathode potential.

† At these concentrations the maximum current was suppressed to about half.

‡ No suppressing effectiveness at these concentrations.

which belonged to divisions 5, 6 or 7 showed almost the same suppressive action for both maxima.

A considerable amount of work[4] has been reported in connexion with the suppression of polarographic maxima, and a number of mechanisms have been proposed. It may be considered that the maximum suppressing effectiveness of the organic addition agent was dependent on its ability to be adsorbed on the electrode, and thus the brighteners of the first class may be considered to be adsorbed on the electrode only at the potential of the positive side of the electrocapillary zero, and to be scarcely adsorbed at the potential of the negative side of the electrocapillary zero. The fact that the brighteners of the first class had a small effect on the cathode potential which was caused by their adsorption on the cathode surface may be explainable on the basis that they are adsorbed on the electrode mainly at the potential of the positive side of the electrocapillary zero.

On the other hand, the brighteners which belonged to divisions 5, 6 or 7 may be considered to be adsorbed on the electrode even at the potential of the negative side of the electrocapillary zero. This is consistent with the fact that these addition agents had a great effect on the cathode potential.

In the case of the brighteners of the first class, the lowest concentration of benzene sulphonate at which brightening was noted was higher than the others and similarly the maximum suppression point for copper was higher. The other brighteners of the first class had almost the same order of the lowest concentration and of the maximum suppression point for copper. It is suggested that there exists some relationship between the maximum suppression point and the minimum concentration required to brighten the deposit which is dependent on the minimum fractional coverage of the surface.

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Synthesis of 6-O-Acetyl-α-Glucose

β-GLUCOSE pentaacetate in acetone solution can be partially deacetylated by cooled 1 *N* potassium hydroxide solution to yield 6-O-acetyl-α-glucose. This product has a melting point of 146°. It has been identified by analysis, molecular-weight determination, downward mutarotation to +53° and hydrolysis to glucose and acetic acid. Periodate oxidation requires 4 moles of periodate per mole 6-O-acetyl-α-glucose without yielding formaldehyde, but glycolaldehyde as *p*-nitrophenyl ozazone could be isolated as a product of oxidation. 6-O-acetyl-α-glucose cannot be tritylated, but a phenylosazone of it has been prepared, m.p. 171°.

6-O-acetyl-α-glucose has been detected among the metabolites of *Bacillus megaterium*[1,2] and has been prepared synthetically[2–4] by the partial acetylation of glucose. Our new synthesis is superior as to yield (20 per cent of theoretical) of the crystalline product.

6-O-acetyl-α-glucose could also be detected among the products of the interaction of tetraacetyl glucosyl bromide and mercuric cyanide followed by deacetylation. This reaction will be described elsewhere.

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Periodic Classification of Elements

To the three requirements of a modern Periodic Table, as stated by A. A. Clifford[1], must be added a fourth, namely, that it should be in as close accord as possible with chemical facts, and above all with the most fundamental of these, valency. It is only in Group IV that there is any strong chemical support for the sub-group arrangement. In other groups the only real justification is that the members of both sub-groups have the same maximum valency and some have the same characteristic valency. In Clifford's table even this slender justification is

destroyed by the inclusion of Pr with Pa, Nd with U, Sm with Pu and Eu with Am, not merely in the same group but also in the same sub-group *c*. Advocates of 'long' forms of table are unlikely to be converted in this way.

It is also wrong to speak of Mendeleef's 'classification' and to identify it with the 'short' form of table. In 1871 Mendeleef himself produced a 'long' form (though he had the 'typical' elements in one block in the centre of the stage instead of in the wings as in most modern tables). In his Faraday lecture of 1889[2], he said: "It [geometrical analysis] must find the means of representing in a special way not only such long periods as that comprising, K Ca Sc Ti V Cr Mn Fe Co Ni Cu Zn Ga Ge As Se Br but short periods like the following: Na Mg Al Si P S Cl". In fact, he was the spiritual parent of all subsequent two-dimensional 'tables', whether 'short' or 'long'. Chemical students must not be defrauded of half their inheritance.

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In comparing my arithmetical table[1] with his adaptation of Mendeleef's short chart, A. A. Clifford[2] states that a Periodic Table must show the variation in the properties of the elements with atomic number. This is an argument against short charts[3], because they fail to retain beyond the third period the continuous variation of properties from active metal to active non-metal. Vertical relationships between the elements are very strong at the extreme right of the arithmetical table; traversing towards the left they weaken, and when the transitional elements are reached horizontal as well as vertical relationships are apparent; still farther to the left, horizontal relationships are most marked[4]. Other tables do not exhibit this important change in direction and strength of the chemical relationships.

Some elements are arbitrarily placed in Clifford's table. A column is headed 3*c*, presumably for symmetry, but it contains no element. Whereas lanthanum is placed in a *d* sub-group, subsequent lanthanides are not so located. The first two actinides are placed in *d* sub-groups, but the rest are in *f* sub-groups. These, doubtless dictated by observed spectrographic ground-states, are arbitrary adjustments within the framework of the table. The provision of two s^1 and s^2 columns in groups 1 and 2 is another arbitrary arrangement.

Clifford's need to choose group 3 as the basis for naming the periods of his table results from the schism that occurs naturally after group 2. This schism is a special feature that distinguishes the arithmetical table from others and follows logically from its mode of construction; each period ends with group 2 and the orbitals are regularly filled.

Short charts are more compact than long, but this is not serious ground for preference. The rectangularity claimed by Clifford is achieved by providing misleading empty spaces at the right of most of his rows. The arithmetical table differs from most other long charts in that it introduces no meaningless medial gaps[5].

Among Luder's arguments against short charts[3] are that no clear separation of metals from non-metals is possible thereby, and that elements which form colourless and diamagnetic ions are not distinguished from those which give coloured and paramagnetic ions.

If Clifford's closing paragraph implies that historical precedence is a factor, the long chart is preferable because Mendeleef's first scheme is stated to be best classed as such[6]. Many well-known modern textbooks are written around long charts[7] and they can scarcely be regarded as an experiment in new forms, whether or not such is to be discouraged.

Among the more important features of the arithmetical table are the logical and didactic advantages of being based on an arithmetical result of quantum principles, and of having a construction that is described succinctly in about two dozen words; most tables defy verbal description and their connexion with basic concepts is obscure. From the form of the table follow a rule for writing down the electronic configurations of atoms in the order of increasing orbital energies, algebraic expressions for calculating the period from the atomic number[1] and the atomic numbers of the members of the vertically related elements[4].

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In my earlier communication[1] I argued that it was better to use a conventional table, adapted to show electronic configurations, than the unusual and reversed forms that have been proposed[2–4]. I chose the short form as giving the best adaptation, pointing out that chemical resemblances between sub-groups are not to be expected.

The conventional long form, adapted and extended to include the inner transition elements, is set out here in Table 1. It has, to a certain extent, the advantages that Simmons claims[2] in showing the change from vertical to horizontal relationships. It shows less clearly, however, which orbitals are being filled in each period. Another disadvantage is that the positions of elements are dictated by the shape of the table, and this is not always desirable. Lanthanum, for example, is in some ways better shown as a transition element. It has no *f* electrons and it shows chemical similarities to yttrium and scandium, while the rare earth elements resemble it. Actinium and thorium are transition elements, electronically speaking; further along the actinide series, because of the small energy difference between the $5f$ and $6d$ orbitals, some chemical properties typical of transition elements are shown. While no generally accepted conclusion can be reached about such elements, my original table has alternative positions

Table 1. Conventional Long Form of Periodic Table adapted to show Electronic Configurations

Periods	Groups	0	1a	2a	Inner transition elements														3a	4a	5a	6a	7a	8a	9a	10a	1b	2b	3b	4b	5b	6b	7b
	Configurations		s^1	s^2	f^1	f^2	f^3	f^4	f^5	f^6	f^7	f^8	f^9	f^{10}	f^{11}	f^{12}	f^{13}	f^{14}	d^1	d^2	d^3	d^4	d^5	d^6	d^7	d^8	d^9	d^{10}	p^1	p^2	p^3	p^4	p^5
1	1		H																													
2	(2*s*)	He	Li	Be																									B	C	N	O	F
3	12	Ne	Na	Mg																									Al	Si	P	S	Cl
4	(3*s* 3*p*) 1 2 $3d^5$ $3p^6$	Ar	K	Ca															Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br
5	(4*s* 3*d* 4*p*) 1 2 3 $4d^5$ $4p^6$	Kr	Rb	Sr															Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I
6	(5*s* 4*d* 5*p*) 1 2 3 $4f^5$ $4d^{10}$ $5d^5$ $5p^6$	Xe	Cs	Ba	La	Ce	Pr	Nd		Sm	Eu	Gd							Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At
7	(6*s* 4*f* 5*d* 6*p*) 1 2 3 4 $5f^5$ $5p^6$ $5d^{10}$ $6d^5$ $6p^6$	Rn	Fr	Ra	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	E	Fm	Md	No															
	(7*s* 5*f* 6*d* 7*p*)																																

which are adjacent. For this reason group 3c which contains no elements is provided.

A Periodic Table is not a law in itself but a way of tabulating (or making generalizations about) facts which are related to more fundamental principles. The advantage Dr. Simmons mentions in (i) having a mathematical definition, (ii) a more regular perimeter, (iii) spaces without alternative positions in them, are considerations of order and presentation of the same importance as, and to be balanced against, practical considerations of convention, observed spectrographic ground-states, and the various chemical relationships.

In my earlier table I used the unconventional system of putting all representative elements in (*a*), and all transition elements in (*b*) sub-groups. The alternative, arising from Mendeleef's original classification, is used in Table 1, and could be used in my original table, though in this case the electronic configurations would follow less simply.

An alternative is to start with each series on a new line as was done in Gardner's chart[5], which has been developed by Luder and Babor[6]. It would be logical to split periods 2 and 3 (Table 1) into two levels also, when the table would resemble an inverted *aufbau* form[4]. Such a table shows clearly the number of the orbital that is being built up in each series and has alternative (non-adjacent) positions for lanthanum, etc. The unconventionality, though slight, of these forms must be the reason they have not been accepted for general use.

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BIOCHEMISTRY

Estimation of 3-Deoxy Sugars by Means of the Malonaldehyde–Thiobarbituric Acid Reaction

During the course of our investigations on the biosynthesis of 3,6-dideoxy-hexoses, found in the endotoxins of *Salmonella* and *Escherichia*[1-3] and in the eggs of *Parascaris equorum*[4], the need arose for a sensitive and specific assay. A consideration of the structures of these compounds suggested to us that the malonaldehyde–thiobarbituric acid reaction, which was adapted by Waravdekar and Saslaw[5] for the estimation of 2-deoxy-ribose, might equally be applicable to the quantitative determination of 3,6-dideoxy-hexoses, and indeed, of 3-deoxy sugars in general. A sample of ascarylose (3,6-dideoxy-L-arabino-hexose), which was the generous gift of Dr. E. Lederer, was used to test this hypothesis.

In the method of Waravdekar and Saslaw, which involved treatment of the sugar with periodic acid at room temperature to yield malonaldehyde, 3-deoxyglucose was found to be virtually unreactive. We have found that ascarylose is similarly unreactive under these conditions. However, by the simple expedient of elevating the temperature of the periodic acid treatment, malonaldehyde was obtained from ascarylose in high yield (Fig. 1). Assuming that the

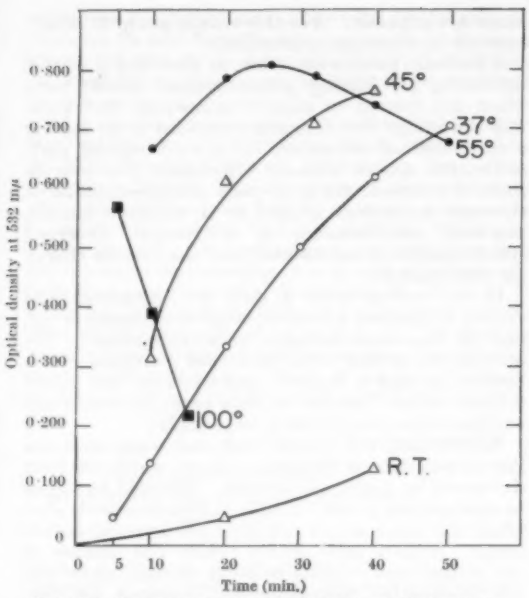

Fig. 1. Reaction of ascarylose in the malonaldehyde–thiobarbituric acid reaction. To each tube, containing 2·62 μgm. ascarylose in a volume of 0·25 ml., was added 0·25 ml. of 0·025 *M* periodic acid in 0·125 *N* sulphuric acid. The tubes were then incubated as indicated in the graph. The reaction was terminated by the addition of 0·5 ml. of 2 per cent sodium arsenite in 0·5 *N* hydrochloric acid. After the iodine colour was discharged, 2·0 ml. of 0·3 per cent 2-thiobarbituric acid were added to each tube, and the samples were placed in a boiling water-bath for 12 min. Optical densities were determined at 532 mμ in a Beckman *DU* spectrophotometer

sample of ascarylose was 100 per cent pure, treatment with periodic acid for 25 min. at 55° gave rise to 88 per cent of the theoretical yield of malonaldehyde. Afterwards, the isolation of abequose (3,6-dideoxy-D-xylo-hexose) from *Salmonella typhimurium* strain *LT*-2, and colitose (3,6-dideoxy-L-xylo-hexose) from *Escherichia coli* strain 0111, permitted us to test these sugars, as well as 3-deoxy-glucose, in our modification of the malonaldehyde–thiobarbituric acid reaction. It can be seen (Fig. 2) that treatment with periodic acid for 20–25 min. at 55° was optimum for malonaldehyde yield.

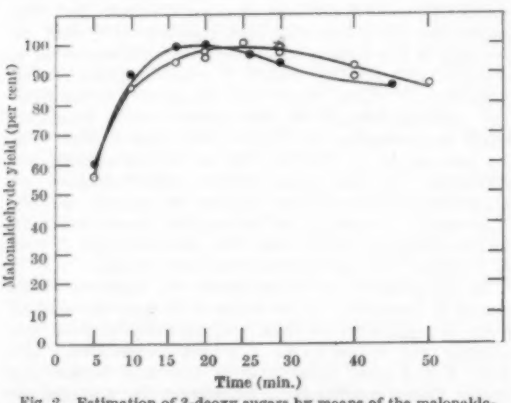

Fig. 2. Estimation of 3-deoxy sugars by means of the malonaldehyde–thiobarbituric acid reaction. The procedure is described in Fig. 1. Temperature of periodic acid treatment was 55°. Each tube contained approximately 0·01 μmole of sugar. ○—○, ascarylose, abequose; ●—●, 3-deoxy-glucose, colitose

With the use of the modification described above of the malonaldehyde–thiobarbituric acid reaction, the assay of 3-deoxy sugars is linear for quantities of 0·001–0·04 μmole. Should even greater sensitivity be required, the solvent-extraction procedure of Waravdekar and Saslaw may be employed. It is of interest to note that the thiobarbituric acid spray reagent of Warren[6], modified for use with sialic acid, has permitted us to detect μgm. quantities of the 3,6-dideoxy-hexoses on paper chromatograms.

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A Novel Synthesis of Polypeptides

We wish to report the formation of polyglycines by direct condensation of glycinamide as an example of the synthesis of linear polypeptides by polymerization of unsubstituted α-amino-acid amides.

Pure glycinamide, melting point 65–66° C., was prepared by the method of Bergell and Wülfing[1]. Alternatively, glycinamide was prepared by other methods[2], or the commercially available glycinamide hydrochloride was directly used after mixing with ammonium hydroxide. The polycondensation reaction involved essentially heating the compound alone, or the hydrochloride together with half its weight of an ammonium hydroxide solution, at 100° C. in a closed vessel for several hours. The product of the reaction was dialysed for four days until no traces of compounds of small molecular-weight remained and then it was lyophilized.

The average molecular weights were determined by a modification[3] of the Van Slyke amino-nitrogen method, which is considered the most reliable method for polyamino-acids[4]. Alternatively, the polymer was dissolved in 60 per cent aqueous lithium bromide and titrated by the Sørensen formol method. Table 1 gives the yields and average degrees of polymeri-

Table 1. Degrees of Polymerization and Percentage Molar Yields of Polyglycines obtained by heating Glycinamide at 100° C.

Reactant and additions	Average degree of polymerization	Percentage molar yield
2 gm. $Gly.NH_2.HCl$* + 1 ml. 2 *N* NH_4OH	40	16·5
2 gm. $Gly.NH_2.HCl$* + 1 ml. 8 *N* NH_4OH	29	74·8
$Gly.NH_2$† (not recrystallized)	33	27·4
$Gly.NH_2$ (crystallized, m.p. 65–66° C.)	34	75·0

The samples were heated in closed vessels for 20 hr. or longer. The degree of polymerization of the first two products was determined by the Sørensen formol titration in lithium bromide and the other two by the Van Slyke amino-nitrogen method (ref. 3).
* Glycinamide hydrochloride (Nutritional Biochemicals Co.).
† Glycinamide prepared by the method of Chambers and Carpenter (ref. 2).

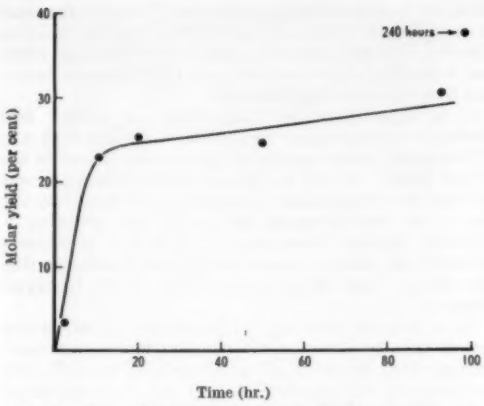

Fig. 1. Rate of formation of polyglycine from glycinamide (ref. 2). Conditions as in Table 1

zation obtained in several experiments, and Fig. 1 shows a preliminary kinetic study of the polycondensation reaction. It can be seen that the initial reaction-rate is sufficiently fast to be able to compete with side-reactions of hydrolysis and ammonolysis.

Proof of structure was afforded by the infra-red spectra. A Beckman *IR*-4 spectrophotometer and potassium bromide disks containing approximately 0·1 per cent polymer were used. Two broad bands for the NH deformation and CO stretching frequencies, 1,534–1,562 and 1,634–1,648 cm.$^{-1}$, respectively, were observed, which indicated superposition of the bands corresponding to the α- and β-forms of polyglycine. This was confirmed by the existence of additional bands at 1,015 cm.$^{-1}$ (β) and 1,028 cm.$^{-1}$ (α), and a shoulder at 1,690 cm.$^{-1}$ (β) (ref. 4).

The products are white or slightly tanned powders, insoluble in water but soluble in concentrated aqueous lithium bromide, as is the case for some of the fibrous proteins. They give a positive biuret test and yield glycine, exclusively, upon acid hydrolysis.

On the basis of these experiments and assuming that most of the α-amino-acid amides can polymerize under similar liquid-phase or vapour-phase conditions, a simple mechanism for the formation of polypeptides on the primitive Earth can be visualized. It requires temperatures accepted by geochemical evidence[5] and uses as reactants logical intermediates in primordial syntheses of amino-acids[6].

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Enzymic Oxidation of Stilbœstrol labelled with Carbon-14

Studies[1–6] on the metabolism of stilbœstrol (or hexœstrol) in animals have failed so far to reveal any significant changes in the œstrogen molecule, apart from conjugation, and *in vivo* a considerable proportion of administered stilbœstrol is excreted unchanged in the urine and fæces. This synthetic œstrogen is used in relatively large amounts for the fattening of livestock and if, after excretion, it were not metabolized by plants or soil micro-organisms, it would tend to accumulate *in situ*, being very insoluble in water, and cause reproductive disorders in animals feeding in that area.

The present communication deals with the conversion of stilbœstrol to water-soluble ether-insoluble metabolites by plant tyrosinase (polyphenol oxidase), an enzyme occurring in mushrooms and many other plants.

[Monoethyl-1-^{14}C]diethylstilbœstrol (3,500 counts/min. in 20 μgm.) was incubated in 5 ml. phosphate buffer (*p*H 6·8) for 5 hr. at room temperature with either a mushroom extract or a tyrosinase preparation, under different experimental conditions, and the amount of radioactivity remaining in the aqueous medium after extraction with ether was determined.

The results in Table 1 show that mushroom tyrosinase is able to attack stilbœstrol with the formation of water-soluble ether-insoluble metabolites in high percentage yields. The reaction was abolished under anaerobic conditions and there was inhibition by a biological reducing agent, such as ascorbic acid, or by potassium cyanide, which forms a complex with the copper in tyrosinase, but iodoacetate, which reacts with sulphydryl groups, had no effect. Protein increased the yield of water-soluble products when less than optimum amounts of tyrosinase were used and it was found that most of this carbon-14 was non-dialysable and accompanied the precipitate formed when trichloracetic acid was added.

The results suggest that mushroom tyrosinase acts on stilbœstrol in the same manner as on its normal phenolic substrates with the formation of highly

Table 1. Percentage Conversion of Stilbœstrol labelled with Carbon-14 to Water-soluble Ether-insoluble Metabolites by Mushroom Tyrosinase under Different Experimental Conditions

Experimental conditions	Weight of tyrosinase incubated (mgm.)	Percentage of added radioactivity remaining in aqueous medium after extraction with ether
		62·3
Incubated for 5 hr.	2	71·0
		67·0
Incubated for 24 hr.	2	78·3
Tyrosinase solution boiled	2	0·9
		0·6
Anaerobic incubation	2	4·4
Incubated with KCN (0·001 *M*)	2	1·5
Incubated with ascorbic acid (0·003 *M*)	2	9·5
Incubated with iodoacetate (0·01 *M*)	2	64·3
Incubated for 5 hr.	0·3	15·5
Bovine serum albumin (10 mgm.) added	0·3	20·3
Egg albumin (10 mgm.) added	0·3	30·2
Gelatin (10 mgm.) added	0·3	32·5
γ-Globulin (10 mgm.) added	0·3	45·8
Human serum (0·2 ml.) added	0·3	44·4
Bovine serum albumin (10 mgm.) added; tyrosinase omitted	—	0·2
Incubated with mushroom extract	—	26·0
Incubated with boiled mushroom extract	—	0·8

reactive *o*-quinonoid derivatives[7] which can then combine with protein or undergo alternative reactions.

The water-soluble metabolites showed no œstrogenic activity when injected into ovariectomized rats at ten times the median effective dose of stilbœstrol.

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Increase in 'Alkaline' Ribonuclease of Rat Kidney Cortex after Lysozyme and Egg-White Administration

Previous work from this laboratory suggested that plasma 'alkaline' ribonuclease is filtered through kidney glomeruli and re-absorbed and inactivated by the tubules[1]; in support of this hypothesis a correlation was found in normal rats between plasma and kidney cortex ribonuclease activity[2]. In the present investigation foreign proteins[3] were administered to rats in an attempt to influence the kidney cortex enzyme activity.

Albino rats of the Wistar strain, 100–200 gm. body-weight, of both sexes were used. Single injections of proteins dissolved in 0·85 per cent sodium chloride were given intraperitoneally in 5–10-ml. volumes. Control rats received only saline. Under ether anæsthesia kidney cortex biopsies (weighing about 25 mgm.) were obtained, one before and the others at different time periods after the injections. 0·5 per cent homogenates were prepared in 0·05 *M* citric acid, neutralized with an equal volume of 0·25 *M* dipotassium hydrogen phosphate, and assayed at *p*H 7·3[4]. Each assay was performed in triplicate on 0·17 mgm. (fresh weight) of tissue. Results are expressed as percentage of ribonuclease activity of the initial biopsies.

Table 1 shows that the administration of egg white or of lysozyme (twice crystallized from egg white) promoted an increase in activity of ribonuclease of kidney cortex after 24 hr.; at 72 hr. the activities fell to initial levels. In other experiments lysozyme was effective at the dose of 0·5 mgm./gm., but not at 0·25 mgm./gm. body-weight. Crystalline egg albumin, bovine albumin (fraction V) and saline had no significant effect on the kidney cortex enzyme activity. It may also be mentioned that egg white and lysozyme administration did not influence serum and liver ribonuclease activity.

Table 1. Action of Foreign Proteins on Ribonuclease Activity of Renal Cortex

Protein	No. of animals	Dose	Ribonuclease activity 24 hr. after injection*	Ribonuclease activity 72 hr. after injection*
Lysozyme	11	1 mgm./gm.	206·2 ± 26·8	123·1 ± 13·2
Egg white	12	4 ml./100 gm.	143·0 ± 8·8	97·8 ± 10·9
Egg albumin	6	3 mgm./gm.	100·2 ± 7·5	102·7 ± 11·4
Bovine serum albumin	6	3 mgm./gm.	110·9 ± 12·6	105·7 ± 11·4
Saline	9		94·1 ± 12·6	90·2 ± 9·0

* As percentage of initial values. Mean ± standard error.

It is interesting to note that egg white[3] and lysozyme (unpublished results with P. Gambetá and J. Gonzalez) easily promote droplet formation in the tubule cells. At 72 hr. the droplets disappear and activity of ribonuclease returns to the initial levels. The other two proteins are much less effective in inducing droplet formation[3]. It is not considered probable, in view of quantitative considerations, that the effect of egg white is ascribable to its lysozyme content.

In a general way it is conceivable that kidney cortex ribonuclease activity could be increased through the operation of one or more of the following mechanisms (cf. also refs. 2 and 3): (*a*) heightened reabsorption, which could be due to increased plasma enzyme content, glomerular filtration, or reabsorptive activity of the tubular cells; (*b*) decreased inactivation of the enzyme by the tubular epithelium, either by failure of the catabolic mechanisms or by a decrease in the activity of the ribonuclease inhibitor[5]. Of these mechanisms only increased serum ribonuclease activity could be ruled out. Further work is needed before a definite choice can be made between these possibilities. Increase in 'acid' ribonuclease activity measured at *p*H 5·0 of rat kidney homogenates after egg white administration has been reported by Strauss[6]. It remains to be shown whether 'alkaline' ribonuclease significantly contributes to the activity at that *p*H.

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Heterogeneous Labelling of Acid-soluble Nucleoside 5′-Monophosphates during Short *in vivo* Incubation with Phosphate-^{32}P

A probable explanation of the heterogeneous ^{32}P-labelling of 5′-nucleotide units of ribonucleic acid of rat liver[1] and deoxyribonucleic acid of thymine-requiring *E. coli*[2] after brief incubation with phosphate-^{32}P is that the carbon-bound phosphate groups of postulated precursor 5′-nucleotides[3] become labelled at unequal rates. Brumm, Potter and Siekevitz[4] have studied the incorporation of phosphorus-32 into acid-soluble nucleotides of rat liver, but the isolated guanylic and uridylic acids were noted to contain impurities. Because there is little direct *in vivo* evidence that 5′-nucleotides actually are the building blocks in biosynthesis of nucleic acids in mammalian tissues, we have re-investigated the status of labelling after a short time of incubation,

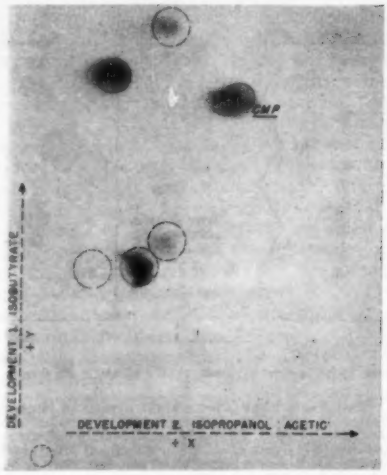

Fig. 1. Radioautograph illustrating problem of radiochemical contamination in short-time experiments with phosphorus-32 and the final purification procedure for nucleoside 5′-monophosphates. Sample applied to origin at lower left: cytidylic acid fraction from ion-exchange purification step of the 20-min. experiment. Development 1, 18 hr. descending. Development 2, 45 hr. descending. Solid lines outline the areas found to absorb light of 254 mμ. CMP identifies the isolated deposit of 5′-cytidylic acid; X = 14·5 cm., Y = 26·5 cm. Its radioactivity is 20 per cent of total. The other deposits are unidentified trace compounds of relatively high specific activity that were eluted from the column with the cytidylic acid

when unequal synthetic rates should be most easily observable.

Three experiments were designed to differ only in incubation time. Four 200-gm. rats of the Long-Evans strain were fasted 17 hr., then injected with about 1·5 mc. $Na_2H^{32}PO_4$ per animal. After equal incubation periods, livers were excised, pooled, and homogenized in 0·3 N perchloric acid at 0° C. Centrifugation yielded the acid-soluble supernatant fraction. The nucleotides in it were adsorbed on 'Norite' and eluted[4]. The eluate was concentrated *in vacuo* and applied as a line to Whatman 3 MM chromatographic paper. Development for 1 day, descending in *iso*propanol/acetic acid/water (60 : 30 : 10), yielded crude fractions I (nucleoside polyphosphates and some trailing guanylic acid) and II (nucleoside monophosphates including fast part of guanylic acid)[5]. Borders of fraction II were outlined with an ultra-violet fluoroscope, and the dividing line between I and II was confirmed by localizing nearly immobile fraction I by high radioactivity (radioautograph) and the guanylic acid by fluorescence[6]. Fraction II was eluted and fractionated by gradient elution from a 'Dowex-1' ion-exchange column[7]. For final purification, peak tubes of formic acid eluate of each nucleotide peak were concentrated *in vacuo*, and the concentrate was applied in duplicate to papers for two-dimensional chromatography. The papergrams were developed in an *iso*butyrate solvent + Y and the *iso*propanol/acetic acid/water solvent + X (Fig. 1; see also ref. 5).

Radiochemical purity was established by parallelism of radioactivity and chemical quantity in serial fractions determined by measurements on intact papergrams[8]. In the 20-min. experiment, radioautographs prepared following additional chromatographic and electrophoretic fractionations further testified to absence of contaminants. As illustrated in Fig. 1, purification carried through only the ion-exchange step by no means sufficed to yield radiochemically pure nucleoside 5′-monophosphates: only adenylic acid was found to be practically pure at that stage.

For quantitative determination of specific activity, radioactivity of a nucleotide was measured on a 1·5-cm. diameter circle cut from the centre of the isolated deposit in a papergram. The nucleotide was then eluted and its chemical quantity determined spectrophotometrically.

Results in Table 1 shows that there is marked heterogeneity of labelling among carbon-bound phosphate moieties of the different 5′-nucleotides of rat liver 20, 30 and 60 min. after injection of phosphate-^{32}P. Heterogeneity is greatest at shortest time, when specific activity of adenylic acid is 8–23 times higher than the specific activity of other nucleotides. There is a trend towards equal labelling by 60 min., when the specific activity of adenylic acid is 5–9 times higher than that of other nucleotides. This naturally occurring selective ^{32}P-labelling of intracellular adenylic acid at short time should offer a unique opportunity for investigating its role as a precursor in the biosynthesis of ribonucleic acid of liver *in vivo*. Leibman and Heidelberger[9] have demonstrated that there is a problem of the impermeability of the cell wall in applying the earlier investigations typified by supplying ^{32}P-labelled adenosine 5′-phosphate in extracellular medium and examining the distribution of phosphorus-32 among nucleotides of intracellular ribonucleic acid. Published work involving phosphate-^{32}P as precursor in rat liver has already indicated that adenylic acid is the most highly labelled 5′-nucleotide unit of each of several cytoplasmic ribonucleic acid fractions after short incubation time[1,10]. Further evidence is being obtained by investigation of nucleotides of the acid-soluble fraction, nuclear ribonucleic acid, and cytoplasmic ribonucleic acid of the same tissue sample.

Table 1. Labelling of Theoretical Precursors of Nucleic Acid after Short Incubations with Phosphate-^{32}P

5′-Nucleotide	Specific activity relative to adenylic acid*		
	20 min.	30 min.	60 min.
Adenylic acid	100	100	100
Cytidylic acid	12·1	13·8	15·9
Guanylic acid	10·5	13·4	18·2
Uridylic acid	4·3	5·0	11·4

* Relative specific activity values found for inosinic acid were 106, 124 and 87 at times indicated. Specific activity values of adenylic acid were 15·6, 25·2 and 61·6 per cent when compared to inorganic phosphate of liver at times indicated.

The rapid increase of concentration of phosphorus-32 in adenylic acid must be ascribed to an unusually high rate of synthesis, since the pool of this substance in rat liver is about 4 times as large as that of any other nucleotide considered. Present evidence on nucleotide synthesis has led to a view that all nucleotides of Table 1 have a final common precursor in the form of the 5-phosphate group of 5-phosphoribosyl-1-pyrophosphate. Inosinic acid is the first purine nucleotide synthesized in this scheme[11], and present results support the theory that it is a precursor of adenylic and guanylic acids *in vivo*. On the other hand, the evidence that uridylic acid is a precursor of the cytidine nucleotides[11] is not strengthened by our consistent finding that specific activity of cytidylic acid exceeds that of uridylic acid.

Radioautographs were prepared by P. H. Dix. The investigation was supported by the Division of Biology and Medicine, U.S. Atomic Energy Commission, and the National Institutes of Health, U.S. Public Health Service. K. K. Tsuboi is an established investigator of the American Heart Association.

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Urinary Excretion of a Ninhydrin-positive Compound, probably a Peptide, after Administration of β-Aminoisobutyric Acid to the Mouse

Intraperitoneal administration to mice of aqueous solutions of substantial amounts of certain amino-acids, including *dl*-β-amino*iso*butyric acid, was found to cause a marked increase in the taurine concentration of the urine and also an increased total excretion of this compound[1].

However, ninhydrin-treated paper chromatograms of urine from mice injected with β-amino*iso*butyric acid demonstrated a spot which was in addition to the three spots expected, namely, those caused by taurine, β-amino*iso*butyric acid and an unidentified compound which has been observed routinely in mouse urine (Fig. 1). (This latter unidentified compound corresponded in chromatographic position to the so-called nephrosis peptide of Dent[2].) The first mentioned of the four ninhydrin-positive spots, which is labelled *D* in Fig. 1, was unique as compared to the other extra, single spots on urinary chromatograms encountered in the course of administration of numerous amino-acids to the mouse[1]. This was because it was found to increase steadily in intensity during the first 12 hr. after the administration of the β-amino*iso*butyric acid. The taurine concentration of urine, in contrast, was maximal at 2 hr., as was the β-amino*iso*butyric acid concentration, as judged by

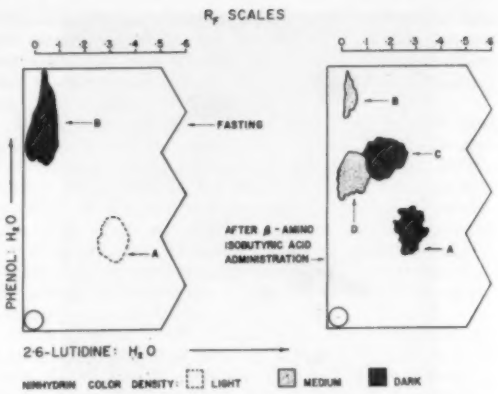

Fig. 1. Two-dimensional paper chromatograms of aliquots from *C3H* mouse urine before (fasting) and about 3 hr. after the administration of 0·8 millimole of *dl*-β-amino*iso*butyric acid intraperitoneally. Areas of colour development after application of ninhydrin are caused by the initial presence of: *A*, taurine; *B*, unidentified compound observed routinely in mouse urine (so-called nephrosis peptide ?); *C*, β-amino*iso*butyric acid; *D*, metabolite of or compound containing β-amino*iso*butyric acid (?). The origin is designated by the circle at the lower left corner in both instances. Phenol/water is the solvent used for the first development. The chromatograms are interrupted in the 2,6-lutidine/water dimension and R_F scales are substituted

inspection of serial two-dimensional paper chromatograms. The simultaneous taurine and β-amino*iso*butyric acid excretion was interpreted to mean that these two compounds were competitively reabsorbed in the kidney tubule. However, the concentration-time relationship of the substance forming spot *D*, on treatment with ninhydrin, suggested that it was a metabolite of, or a compound containing, the β-amino-acid administered.

An attempt was made to prepare the substance in an amount sufficient for identification. It was formed by administering 0·4 m.mole of *dl*-β-amino-*iso*butyric acid intraperitoneally to each of several fasted *C3H* mice. The combined 24-hr. urine samples were applied directly to paper for chromatographic purification. The solvents used for paper chromatography (for preparative, and other purposes herein) were: (1) phenol, saturated with respect to (*a*) water or (*b*) a 10 per cent sodium citrate aqueous solution; (2) formic acid/*t*-butyl alcohol/water (15 : 70 : 15 by volume); (3) 2,6-lutidine/water (65 : 35); (4) *n*-propanol/water (4 : 1). The compound which formed spot *D* when treated with ninhydrin moved predominantly as a single component after numerous chromatographic developments and subsequent selective elutions. However, if it appeared without contamination after exhaustive unidimensional chromatography, the compound was always accompanied by other ninhydrin-positive material on subsequent two-dimensional development with phenol and lutidine solvents. The conclusion was drawn that the compound could not be isolated readily in a pure state by repeated paper chromatography employing the solvents mentioned. Therefore, the compound, after being subjected to preparative paper chromatography, is referred to here as being purified. (The most reasonable explanation of the above findings was that, at times, the chromatographic isolation technique caused a moderately rapid breakdown of the substance. The responsible agents in this technique were probably certain of the chromatographic solvents.)

The purified material was chromatographed unidimensionally with authentic amino-acids on paper by the ascending technique[3]. It did not migrate with an R_F identical to any of the following: histidine, alanine, citrulline, glutamine, β-alanine, arginine, lysine, glycine, glutamic acid, ornithine, β-amino*iso*-butyric acid, glutathione (applied as the oxidized, reduced or sodium salt forms), or carnosine. The purified compound was boiled with 6 *N* hydrochloric acid for 18 hr. Two-dimensional paper chromatograms of the hydrolysate revealed several ninhydrin-positive areas with disappearance of all, or nearly all, the coloration in the area ordinarily occupied by the original compound.

Concomitant and additional attempts to isolate the compound included the following. 'Dowex 50' column chromatography of the partially purified compound with 1–4·5 *N* hydrochloric acid as eluant[4] resulting in great losses presumably due to hydrolysis of the compound. In the one experiment attempted, electrophoresis of the purified compound on filter paper (Whatman 3 *MM*) in a 0·08 *M* ($\mu = 0{\cdot}48$) potassium phosphate buffer at *p*H 6·0 employing a constant current of 20 m.amp. for 6½ hr. in a Spinco model *R* apparatus resulted in the compound migrating toward the cathode as a single band, as visualized after treatment with ninhydrin.

No significant difference in ultra-violet absorbancy was found between eluates from areas on paper chromatograms which did contain and from those which did not contain the compound which, when treated with ninhydrin, formed spot *D*. It was not possible to detect free sulphydryl groups by the nitroprusside test[5]. This test employed an amount of purified compound which would have given colour after treatment with ninhydrin equivalent to about 125 μgm. of alanine.

In summary, the simple means of isolation employed did not yield a demonstrably pure product, although preliminary work with paper electrophoresis indicated a possible method of preparation. Failure to isolate the compound was thought to be due to its lability, rather than to failure to remove contaminating material. It was probable that the compound was a peptide or that it contained several amino-acids. Therefore, considering the method of inducing its production, the possibility exists that β-amino*iso*butyric acid is one of the constituent amino-acids. Relative to the possibility of this occurrence, Westall[6] found β-amino*iso*butyric acid present as a trace amino-acid following acid hydrolysis of some 10 different peptides, grouped together, originally separated from human urine.

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ANIMAL PHYSIOLOGY

Calcium and Magnesium in Human Cerebrospinal Fluid

Little has been added in the past thirty years, as may be seen from Davson's valuable monograph[1], to the pioneer observations of Cohen[2] and of McCance and Watchorn[3] on the magnesium and calcium of human cerebrospinal fluid. In our studies of the blood-cerebrospinal fluid barrier we have made many observations with newer microchemical methods[4], from which some points of interest emerge.

In Table 1 are shown the mean values for calcium and magnesium of the lumbar fluids and blood sera of thirty-eight patients about as nearly normal as obtainable. The bromide distribution ratio, R_{Br} (cerebrospinal fluid/serum bromide), and the protein content of the cerebrospinal fluid support this assumption[5].

Table 2 shows the same fluids arranged in three groups according to the levels of calcium and of magnesium in the sera. It will be seen that neither the calcium nor the magnesium of the lumbar fluids shifts significantly with changes in the serum-levels.

Table 3 shows a group of patients with non-inflammatory neurological conditions along with a group of patients with active tuberculous meningitis. In each group contemporary taps were made at different levels of the neural axis, mostly lumbar and ventricular, but sometimes lumbar and cisternal or ventricular and cisternal. The non-inflammatory group is referred to as 'near to normal'. Its patients show a higher lumbar cerebrospinal fluid protein, a higher R_{Br}, and a rather higher calcium and lower magnesium than the patients in Table 1. The differences in the mean values found for both calcium and magnesium in lumbar, cisternal and ventricular fluids would not appear to be significant especially if one bears in mind the abnormality, just indicated, of the group. We can thus confidently say that there is no gradient for calcium and magnesium in cerebrospinal fluid at different levels of the neural axis of normal people. This contrasts sharply with the marked gradient existing for both protein and bromide.

The activity of the tuberculous meningitis in the remaining group in Table 3 may be judged from the high lumbar bromide distribution ratio of 0·94. This is accompanied by an increase in the concentration of calcium of nearly 20 per cent and a drop in the concentration of magnesium of 25 per cent. The effects of the pathological process are much less marked in the cisternal and ventricular fluids and a gradient for both calcium and magnesium becomes apparent.

In his remarkable paper in 1927, Cohen[2] first observed that there was more magnesium in normal human cerebrospinal fluid than in the corresponding serum, that the magnesium was lowered in tuberculous meningitis to nearly the serum value and that the fluid level was largely independent of the serum level. We endorse those findings though our absolute analytical values are some 20 per cent lower than Cohen's. McCance and Watchorn[6] also found a lowered magnesium concentration in the lumbar fluids of patients with tuberculous meningitis with no significant shift in the calcium concentration. Surprisingly, the same workers[7] found a normal cerebrospinal fluid magnesium concentration in loculated fluids taken from below a tumour and

Table 1. Calcium and Magnesium Concentrations in Serum and Lumbar Fluids of 38 Patients with Mild Neurological Conditions

Statistic	Calcium			Magnesium			Protein in CSF (mgm./100 ml.)	
	CSF (mgm./100 ml.)	S (mgm./100 ml.)	R_{Ca}	CSF (mgm./100 ml.)	S (mgm./100 ml.)	R_{Mg}		R_{Br}
Mean	4·56	9·41	0·486	2·71	1·96	1·386	33·6	0·36
Range	3·9–5·1	8·2–10·6	0·38–0·56	2·40–2·95	1·65–2·35	1·15–1·63	18–64	0·26–0·46
S.D.	0·32	0·64	0·039	0·108	0·134	0·105	—	0·046

CSF, Cerebrospinal fluid; S, serum.

Table 2. Relation between Cerebrospinal Fluid and Serum Calcium and Magnesium—from the Group of Patients in Table 1

Calcium					Magnesium				
Serum interval (mgm./100 ml.)	Number in interval	Mean values for interval			Serum interval (mgm./100 ml.)	Number in interval	Mean values for interval		
		CSF (mgm./100 ml.)	S (mgm./100 ml.)	R_{Ca}			CSF (mgm./100 ml.)	S (mgm./100 ml.)	R_{Mg}
<9·2	15	4·41	8·79	0·502	<1·91	14	2·68	1·83	1·46
9·21–9·60	12	4·74	9·51	0·499	1·91–2·00	15	2·71	1·98	1·37
>9·60	11	4·56	10·21	0·450	>2·00	9	2·73	2·13	1·28

CSF, Cerebrospinal fluid; S, serum.

Table 3. Calcium, Magnesium and Protein Concentrations in Lumbar (L), Cisternal (C), and Ventricular (V) Fluids, with Bromide Distribution Ratios in Patients with Non-inflammatory Neurological Conditions, 'Near to Normal', and in Patients with Active Tuberculosis Meningitis

Group	Number of fluids	Statistic	Calcium (mgm./100 ml.)			Magnesium (mgm./100 ml.)			Protein (mgm./100 ml.)			R_{Br} (mgm./100 ml.)		
			L	C	V	L	C	V	L	C	V	L	C	V
Near to normal	L, 19	Mean	4·65	4·49	4·56	2·66	2·60	2·72	59	36	15	0·41	0·28	0·27
	C, 6	Range	4·1–	4·1–	4·0–	2·30–	2·35–	2·35–	18–	17–	2–	0·22–	0·25–	0·24–
	V, 20		5·5	4·9	5·1	2·95	2·80	3·05	133	54	42	0·55	0·32	0·32
Active tuberculous meningitis	L, 14	Mean	5·4	4·8	4·5	2·02	2·46	2·53	240	78	40	0·94	0·45	0·34
	C, 6	Range	4·7–	4·5–	3·8–	1·45–	2·25–	2·35–	120–	35–	7–	0·81–	0·30–	0·24–
	V, 9		6·4	5·4	5·1	2·40	2·60	2·75	420	129	88	1·10	0·59	0·47

containing much protein. We have analysed many such fluids and invariably found magnesium concentrations near serum values.

Our findings too are in accordance with 'Cohen's law' that in tuberculous meningitis the concentrations of constituents of the cerebrospinal fluid tend to become equal to those of the serum. This conception, however, suggests a relationship between the two fluids that is probably simpler than the reality. If the concentrations of calcium and magnesium in the cerebrospinal fluid are largely independent of the plasma concentrations, as they appear to be, the idea of equality of distribution between the two fluids would appear to have little meaning for those two elements.

On the other hand, the bromide ion concentration in cerebrospinal fluid is in a sensitive balance with that in the plasma, and here we not infrequently find that the distribution ratio markedly exceeds unity. In Table 3 the highest value shown is 1·10 but in other cases we have found it to be more than 1·3: that is, the pendulum has swung far beyond the balance expected from Cohen's law.

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Suppression of Increased Capillary Permeability in Injury by Monoamine Oxidase Inhibitors

Previous work has indicated that many of the vascular phenomena of inflammation (especially vasodilation and increased capillary permeability) may be attributed to the local release or activation of compounds with the appropriate pharmacological properties, for example, histamine, peptides or globulins[1–5]. A possibility that has received little or no attention is that injury may lead to the local destruction of vasoconstrictor compounds, thus enhancing the effect of released vasodilators.

The vasoconstrictors possibly involved would be adrenaline, noradrenaline and 5-hydroxytryptamine. Of these, adrenaline seems the most likely, at least in the rat, because of its powerful effects on capillary permeability[6]. There is more than one pathway for the destruction of these amines in the tissues[7]. One such mechanism is by the action of the enzyme monoamine oxidase. Specific inhibitors of this enzyme exist, and it was decided to test the effect of such compounds in experimental acute inflammation.

Male albino rats were used and the forms of inflammation studied were standard thermal injury (55° for 27 sec.) and pleurisy induced by intrapleural turpentine. Increased capillary permeability to protein was used as a measure of inflammatory changes and was assayed by methods used previously (in thermal injury, water content of skin and subcutaneous tissue in gm./cm.[2]; in turpentine pleurisy, volume of exudate[8,9]).

Table 1

Treatment	Thermal injury. Increase in water content of skin at 3 hr. (gm./cm.²)	Turpentine pleurisy. Volume of exudate (ml.) at 16 hr.
None (control)	0·11	2·7
Iproniazid	0·050	1·4
Iproniazid and dibenamine 8 mgm./kgm. (intravenous)	0·10	—
Iproniazid and adrenalectomy	0·06	—
Adrenalectomy alone	0·14	—

Values represent the means of 12 experiments

Table 1 shows that administration of the monoamine oxidase inhibitor iproniazid (1-*iso*nicotynyl 2-*iso*propylhydrazine) (200 mgm./kgm. subcutaneous) 1 hr. before injury led to striking inhibition of increased capillary permeability in both types of inflammation. Similar results were obtained with four other monoamine oxidase inhibitors, compounds pheniprazine (1 methyl-2 phenylethyl hydrazinium chloride), 1275 ([1-methyl-2 phenoxyethyl] hydrazinium hydrogen malate) and 1273 [(2-4′ hydroxyphenoxyethyl) hydrazinium chloride] and 1325 (2 : 2 [*p*-phenylenedioxy] di(ethyl hydrazinium chloride). The table also shows that administration of the adrenolytic dibenamine (N.N.dibenzyl-*b*-chloroethylamine hydrochloride, 8 mgm./kgm. intravenous) prevented completely the action of iproniazid. Dibenamine had a similar effect on the action of the other monamine oxidase inhibitors, but not of other anti-inflammatory drugs, for example quinine. In addition it can be seen that bilateral adrenalectomy two weeks previously failed to prevent the action of iproniazid. Adrenalectomy alone seemed to raise the response of rats to thermal injury.

A single large injection of adrenaline (0·5 mgm./kgm. subcutaneous) 20 min. before thermal injury led to a reduction of inflammatory œdema of the order seen after administration of iproniazid. Similar injections of noradrenaline or of 5-hydroxytryptamine had no such effect and in higher doses led to the collapse or death of the animals.

Another enzyme that destroys adrenaline in the tissues is catechol-O-methyl transferase[7]. Pyrogallol is an inhibitor of this enzyme[7]. Administration of pyrogallol (200 mgm./kgm. intravenous) 30 min. before thermal injury failed to lessen the resultant inflammatory œdema.

Doses of iproniazid which suppressed inflammatory œdema had no significant effect on the increased capillary permeability caused by intradermal injections of histamine, 5-hydroxytryptamine, substance *P* (the vasoactive peptide), compound 48/80 and serum globulins.

From the above results it is concluded that following injury in the rat there is a local destruction of a vasoconstrictor substance. This destruction is probably effected by the enzyme, monoamine oxidase, and presumably augments the action of the locally released vasodilator compounds. Despite evidence that monoamine oxidase may play only a minor part in the inactivation of adrenaline[7], the present results seem to indicate that the vasoconstrictor compound inactivated in injury resembles adrenaline rather than 5-hydroxytryptamine or noradrenaline. These results will be discussed more fully elsewhere.

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Effect of *p*H on Biological Activity of Pregnant Mare Serum Gonadotropin

Among the factors changing the biological activity of gonadotropins the physico-chemical properties of the injection medium may be of importance. Thus the effect of human chorionic gonadotropin is increased when the hormone is dissolved in serum or in plasma instead of saline[1]. Moreover, certain 'augmenting substances' may change the biological effects of the gonadotropins, quantitatively as well as qualitatively[2].

In our studies we have found that certain differences may be recorded if different media are used for dissolving the same batch of gonadotropin. To elucidate further this problem we have performed systematic investigations on the effect of *p*H on the biological activity of pregnant mare serum gonadotropin. A commercial preparation of this hormone was used throughout ('Gestyl', Organon). White mice of an inbred strain, weighing 9–10 gm., were used as test animals. During the experiment the animals were given standardized food and water *ad libitum*. The pregnant mare serum hormone was dissolved in 0·1 *M* buffers of various *p*H and composition. The following buffers were used: (1) the universal buffer of Teorell–Stenhagen[3]; (2) the citric-acid buffer of McIlvaine; (3) the phosphate buffer of Sörensen; (4) the boric acid buffer of Sörensen. The dose of pregnant mare serum gonadotropin administered was 0·2 I.U. per animal dissolved in 1 ml. of the buffer solution. This volume was divided into three parts which were injected at approximately 24-hr. intervals on each of three consecutive days with necropsy on the fourth day. Uterus and ovaries were weighed on a torsion balance to the nearest 0·5 mgm. Half the animals in each group were injected subcutaneously and the other half intraperitoneally.

The statistical treatment of the results obtained was performed with the usual '*t*'-test. When $\leqq P$ 0·05 the difference found was regarded as statistically significant.

Regarding the two types of injection used it could be demonstrated that there was no significant difference between injections made subcutaneously and intraperitoneally. Thus the two groups injected in different ways were put together and used as a single unit in Table 1.

Table 1

No. of animals	pH	Buffers	Uterine weight (mgm.) (mean ± standard deviation)	Difference
8	4	Teorell–Stenhagen	16·1 ± 7·9	Not significant
8	4	McIlvaine	15·5 ± 7·8	
8	4	Teorell–Stenhagen (controls)	7·9 ± 1·5	
6	4	McIlvaine (controls)	8·3 ± 1·9	
8	7	Teorell–Stenhagen	38·2 ± 6·3	$0·01 > P > 0·001$
8	7	Sörensen (phosphate)	22·9 ± 11·7	
8	7	Teorell–Stenhagen (controls)	6·4 ± 1·5	
6	7	Sörensen (phosphate) (controls)	7·4 ± 1·3	
8	10	Teorell–Stenhagen	26·4 ± 9·9	Not significant
16	10	Sörensen (boric acid)	21·4 ± 7·7	
8	10	Teorell–Stenhagen (controls)	7·6 ± 1·4	
6	10	Sörensen (boric acid) (controls)	7·7 ± 2·3	

No difference could be demonstrated between the two buffer systems used at *p*H 4 and *p*H 10 respectively. At *p*H 7, on the other hand, there was a significant difference between the uterine weight response when pregnant mare serum gonadotropin was dissolved in the universal buffer (Teorell–Stenhagen) and in phosphate buffer (Sörensen) respectively. The reason for this is obscure. Controls, injected with the various buffer solutions only, showed no increase of their uterine weights.

The effect of *p*H on the uterine weight response is shown in Table 2.

Table 2

No. of animals	pH	Buffers	Uterine weight (mgm.) (mean ± standard deviation)	Difference
16	4	Teorell–Stenhagen + McIlvaine	15·8 ± 7·6	$0·01 > P > 0·001$
24	10	Teorell–Stenhagen + Sörensen (boric acid)	23·0 ± 8·1	
16	4	Teorell–Stenhagen + McIlvaine	15·8 ± 7·6	$P < 0·001$
8	7	Teorell–Stenhagen	38·2 ± 6·3	
16	4	Teorell–Stenhagen + McIlvaine	15·8 ± 7·6	$0·1 > P > 0·05$
8	7	Sörensen (phosphate)	22·9 ± 11·7	
24	10	Teorell–Stenhagen + Sörensen (boric acid)	23·0 ± 8·1	$P < 0·001$
8	7	Teorell–Stenhagen	38·2 ± 6·3	
24	10	Teorell–Stenhagen + Sörensen (boric acid)	23·0 ± 8·1	Not significant
8	7	Sörensen (phosphate)	22·9 ± 11·7	

Clearly enough, pregnant mare serum gonadotropin preparation showed its highest and optimal effect at *p*H 7. At *p*H 4 the effect was a rather low one. At *p*H 10 the hormone showed a fairly high effect, significantly higher than at *p*H 4.

Concerning the ovaries, the dose is too low to give any measurable effect on the weight of this organ. On the other hand, our studies gave certain evidence for the existence of qualitatively different effects of pregnant mare serum gonadotropin on these organs at different *p*H. Studies on these problems are in progress.

During the preparation of this communication it has come to our knowledge that a similar investigation on the effect of *p*H on the activity of human chorionic gonadotropin has been performed[4].

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Aortic, Serum, Connective Tissue and Osseous Chemistry in Lathyritic Rats

In several previous papers we have reviewed aspects of the pathogenesis of arterial lesions in man and in experimental animals with particular reference to the frequently reported increases in histochemically demonstrable material, taken by microscopists to be mucopolysaccharides[1–3]. In an attempt to determine the significance of this particular finding in the pathogenesis of degeneratory arterial diseases we also studied the vascular lesions induced in rats by *Lathyrus odoratus* intoxication, in which mucopolysaccharide increases have been noted by several authors besides us[4–6]. All these investigators considered the increases in mucopolysaccharides to be derived directly from increased rates of lysis of vascular components and/or depolymerization of aortic ground substance, attendant on *Lathyrus* intoxication. Our own view, based on histochemical and aortic growth studies, is that the lesions in lathyrism may perhaps be due to decreased synthesis of the elastin, and other components of vascular elastic membranes, associated with normal rates of lysis attendant on the growth of the rat's aorta during the period of maximal vascular sensitivity to the toxin[2,7].

Essential factual requirements in support of either of the above views would seem to include a knowledge of: (*a*) whether the lesions are indeed associated with any chemically demonstrable alterations in elastin or collagen content of aortic tissue, and (*b*) whether, indeed, the histochemical observations are a true reflexion of the mucopolysaccharide content of the aorta of *Lathyrus*-intoxicated rats. To our knowledge no one has yet provided these facts so essential for a correct understanding of the genesis of lathyritic and perhaps also of other vascular lesions.

With the view of providing these facts, twenty weanling albino, Wistar strain male rats, initially 21 days old (30 gm.), were placed on a diet consisting of 50 per cent ground *L. odoratus* seeds and 50 per cent of the normal stock diet. Rats of this age are considered essential in view of our previous finding that this species is maximally sensitive to vascular lesions during the period of rapid post-natal aortic growth[2,7]. Twenty similar controls were fed the stock diet alone. After 25 days, all rats were killed by ether anæsthesia and bled from the heart. The several tissues removed for analysis (Table 1) were dehydrated with acetone, defatted with ether/alcohol (1 : 1) and dried to constant weight. The respective tissues and sera of the ten rats in each group were pooled, thus giving two samples for each group. All chemical

Table 1

	Percentage of dry fat-free tissue								Mgm./100 ml.	
	Aorta		Skin		Tail tendon		Femur		Serum	
	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.
Hexose	0·77	0·75	0·51	0·60	0·70	0·65	0·39	0·40		
Hexosamine	0·53	0·29	0·22	0·23	0·27	0·31	0·17	0·14		
Uronic acid	0·72	0·52	0·25	0·22	0·22	0·27	0·26	0·28		
Hydroxyproline*	2·73	2·70	6·64	5·38	7·18	7·63	1·69	1·81		
Elastin	33·8	35·3								
Calcium	0·30	0·35	0·15	0·18	0·70	0·82	21·3	22·6	11·6	11·5
Mucoprotein									216	158

Exp., Rats consuming 50 per cent *L. odoratus* diet; Con., rats consuming stock diet. Mean of 20 rats in each of the experimental and control groups.
* Extractable by autoclaving.

analyses were carried out in duplicate using methods already described[8] except for uronic acid, which was estimated according to the method of Dische[9].

It was found (Table 1) that in the *Lathyrus*-treated animals there was a marked rise in the aortic hexosamine and uronic acid contents, this being consistent with the increase in metachromatic and ferrocyanide-positive material shown histochemically by several investigators (see above). We have also confirmed in rats Schwartz's finding[6] (in rabbits injected with nitriles) of large increases in serum mucoprotein-levels. However, neither the serum nor tissue calcium-levels were altered in any of our rats. Nor did the collagen and elastin contents of the aortæ or other tissues of the treated animals differ strikingly from those found in our controls. Moreover, no notable changes in composition occurred in the skin, tail tendon or femur of lathyritic animals, except for a suggested increase in cutaneous hydroxyproline.

Our findings thus confirm chemically the frequently repeated histochemically demonstrable increase in aortic mucopolysaccharide content, while indicating that a similar increase in mucopolysaccharide is not demonstrable in the other connective tissues here studied. Thus, the increased depolymerization of aortic and other tissue components, claimed by others to be the basis for increases in both aortic and serum mucopolysaccharides, are not supported by our findings, especially in view of the absence of any alteration in elastin and collagen contents associated with detectable increase in mucopolysaccharide.

It therefore seems probable that the elevated aortic and serum mucopolysaccharide-levels in lathyrism may not be due to the destruction of the components of aortic vascular elastic membranes as seems to be the case in calciferol-intoxicated rats[8] or, as suggested by Schwartz and Gilmore[10], for human atherosclerosis. The possibility still exists, therefore, that the increase in serum and aortic mucopolysaccharides in lathyritic rats may be due to increased synthesis and discharge of mucoproteins (by the liver and probably other tissues) associated with a decreased rate of utilization of mucopolysaccharide by the aorta and other connective tissues during the synthesis of new fibrillar components. This possibility is at least in conformity with our previous observations that serum mucoproteins may be elevated in association with either the synthesis or the degradation of various connective tissue components[11,12].

The accumulation of mucopolysaccharide around and between the vascular elastic membranes of lathyritic rat aortæ may, as previously suggested by us, thus indeed be merely one expression of some block in the neogenesis of the elastin and collagen-like fibrillar components of vascular elastic membranes during the early post-natal period of active aortic growth[3,7], perhaps analogous with the block in collagen synthesis in healing wounds in scorbutic guinea pigs. However, we believe that a final answer to this problem can be anticipated only from suitably planned radioactive tracer studies, including an auto-radiographic analysis of the rate of turn-over of 'labelled' aortic membranes in rapidly growing animals.

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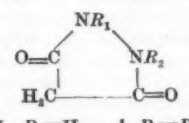
Antipyretic Action of *l*-Phenyl-3,5-pyrazolidinedione

Buchi *et al.*[1] have recently studied the pharmacological properties of a series of substituted 3,5-pyrazolidinediones (I). A substituting group in the 4-position influences these properties more profoundly than does the same group in the 2-position. The authors have reported that *l*-phenyl-3,5-pyrazolidinedione (I*a*) (*LD*50 is approximately 1,600 mgm./kgm. in mice) possesses nearly double the analgesic action of the 1,2-disubstituted derivative, namely, 1,2-diphenyl-3,5-pyrazolidinedione (I*b*) (*LD*50 is 800 mgm./kgm. in mice). However, the authors did not report the antipyretic activity of I*a*.



I*a*, R_1=H ; R_2=H *b*, R_1=R_2=C_6H_5

Table 1. Testing the Antipyretic Action of *l*-Phenyl-3,5-pyrazolidinedione (Ia) on Rabbits
(Dose, 100 mgm./kgm. body-weight by stomach tube)

Rabbit	Weight (gm.)	Normal rectal temperature (° C.)	Temperature before administration	1 hr. after giving drug	2 hr. after	3 hr. after	Sum of differences	Mean
A	900	38·9	40·6	39·9	39·3	39·7	2·9	2·9
B	970	39·0	40·0	39·0	39·0	39·2	2·8	
C	1,150	39·2	40·1	39·4	39·3	39·0	2·6	
D	1,450	39·0	40·9	39·6	39·3	39·4	4·4	
E	1,450	39·1	40·9	40·5	40·3	40·4	1·5	
F	1,500	39·1	41·2	40·4	40·2	39·8	3·2	

Table 2. Testing the Antipyretic Action of Aminopyrine on Rabbits
(Dose 100 mgm./kgm. body-weight by stomach tube)

Rabbit	Weight (gm.)	Normal rectal temperature (° C.)	Temperature before administration	1 hr. after giving drug	2 hr. after	3 hr. after	Sum of differences	Mean
A	1,000	38·6	40·3	38·8	38·7	38·7	4·8	3·32
B	1,170	39·2	40·4	39·8	39·5	39·5	2·4	
C	1,300	38·8	40·9	39·3	39·1	39·2	5·1	
D	1,450	39·1	40·9	40·9	40·4	40·8	0·6	
E	1,670	39·4	40·8	39·7	39·7	39·3	3·7	

We would like to report the antipyretic action of I*a*. The method adopted was that of Brownlee[3]. Animals weighing between 1 and 2 kgm. were selected and kept without food, though given water. *T.A.B.* vaccine was used intravenously in the ear vein in a dose of 2 ml./kgm. of the undiluted vaccine. The rise of temperature was evident 1 hr. after the injection, and after 2 hr. the temperature was sufficiently steady for the antipyretic test to be carried out. Aminopyrine was taken as a standard for comparison. The results are tabulated in Tables 1 and 2. The temperature was recorded hourly for 3 hr. after giving both compounds.

The two figures for the mean of the sum of differences indicate that I*a* is 87·3 per cent as active as aminopyrine as an antipyretic agent.

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Trehalose Metabolism of Trypanosomes

The trypanosomes causing African sleeping sickness from the blood of man and other animals can be cultivated on non-living media. Such cultures exhibit one striking peculiarity: as soon as established they are not infectious for mammals.

I have reported that, when trehalose is added to blood agar medium, infectious cultures of *Trypanosoma rhodesiense* are obtained, indicating that trehalose participates in the control of infectivity in this system[1].

It is now known that, in natural circumstances, trehalose is available to the trypanosomes within their insect hosts. Tissue analyses were performed on *Glossina palpalis* and *G. brevipalpis*, vectors of human trypanosomiasis captured in the field in Africa. The trehalose content of the intestine ranged from 10 to 19 mgm./100 gm. of tissue[2]. Trypanosomes migrate extensively within *Glossina*, during which migration they lose and then regain infectivity. Exposure to trehalose during the non-infectious intestinal phase is of interest as possibly relating to the ultimate regain of infectivity.

Strains of both *T. gambiense* and *T. rhodesiense* have now been found which are activated by trehalose. It has also become clear that trehalose is not the only factor which controls infectivity; for strains sensitive to trehalose suddenly lose this sensitivity after passage. This loss has taken place with a strain isolated directly from man after six subcultures and a total period *in vitro* of 69 days, and more rapidly with strains received after passage in animals. Apparently, at least two factors are concerned. One is known: trehalose; the other or others are responsible for the utilization of trehalose, and remain to be identified. Thus far only strains recently isolated from man have proved sensitive to trehalose: conversely, old laboratory strains are usually insensitive. The practical problem of having suitable strains to work with may be met, at least in part, by storage of the trypanosomes at −70° C. and thus avoiding passage. Such material has been activated by trehalose after 104 days at −70° C.

Loss of sensitivity to trehalose is one more example of the extreme lability of *T. rhodesiense* and *T. gambiense*, for it is already known that during laboratory maintenance in animals these trypanosomes lose their cultivability[3,4] and become unable to develop in *Glossina*[5,6].

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Giant-Fibre Reflex of the Earthworm

THE rapid shortening of an earthworm is co-ordinated by giant fibres located in the nerve cord[1,2]. Though the transmission characteristics and anatomy of these giant internuncials are established, their afferent and efferent connexions are unknown[3–5].

An outstanding feature of the shortening response is its rapid fatigue on repetition. On analysis there are three points in the reflex pathway where failure of transmission might occur. These are: (*a*) between the motor neurones and the muscle cells, the neuromuscular junction; (*b*) between the giant internuncial and the motor neurones, the giant-to-motor junction; and (*c*) between the sensory neurones and the giant internuncial, the sensory-to-giant junction. No evidence has suggested that a physiological junction occurs in the course of the motor nerves, at least for the rapid responses.

The neuro-muscular junction was investigated by repetitively stimulating the central end of a segmental nerve and recording the action potentials produced in the longitudinal muscle of that segment. At a frequency of 4 per sec., each shock, including the first, produces an action potential of which the first two or three show a progressive increase in size. Thereafter the size of the potential is maintained for about 80 shocks after which it gradually decreases in magnitude. The decline, however, is very slow and after 700 shocks the potential is only reduced to half its original size. It requires several hundred more shocks to cause the response to disappear altogether. The recovery time is short, and after a rest period of 2 min. the muscle potential returns to its original size and responds to the first shock. It is therefore clear that the neuro-muscular junction transmits impulses in a one-to-one manner for a considerable time and fatigue of the rapid response must be located elsewhere.

The giant-to-motor junction has proved more difficult to investigate because it is usually stimulated to failure during the dissection. However, action potentials may be recorded from the longitudinal muscle in response to impulses in the median and/or the lateral giant fibres if the operation is performed sufficiently quickly. A short length of the nerve cord was exposed at the posterior end of the worm. The anterior end of the nerve cord was then directly stimulated through the mid-ventral body wall with repetitive shocks and the giant-fibre impulses so produced were recorded from the posterior end. Action potentials were recorded simultaneously from the longitudinal muscle. The results vary greatly from preparation to preparation and also from one region of the muscle to another. Usually accommodation of the giant-to-motor junction was already complete, no muscle potentials being initiated by giant-fibre impulses. However, in favourable preparations the first few shocks are followed by muscle potentials after a delay (25 millisec.) which is so short that the response can only have arisen from the giant-fibre impulses. The junction transmits for a short while in an irregular manner and thereafter no further muscle potentials are recorded unless stimulation is stopped and the preparation allowed to recover. The muscle potentials vary in size as they also do when shocks of varying intensity are applied to a segmental nerve, observations which suggest that in each segmental nerve there are several motor fibres each with its own giant-to-motor junction. Physiological evidence indicates that there are three motor neurones, or groups of motor neurones, innervating the longitudinal muscle in the first segmental nerve, and at least two, probably three, in the 'double nerve' (the second and third segmental nerves). This has not yet been confirmed histologically and the morphological relation between the motor neurones and the giant internuncials is uncertain.

The sensory-to-giant junction also accommodates rapidly and again the operation must be designed to avoid excessive shock. If one of the segmental nerves is stimulated through the ventral body wall at the anterior end, median giant fibre impulses can be recorded from the nerve cord exposed at the posterior end. In the majority of preparations the sensory-to-giant junction accommodates after the first one or two shocks at three per second and remains accommodated for as long as the segmental nerve is stimulated. If, however, stimulation is stopped the junction recovers and is found to transmit again after a period of between one and three seconds. By stimulating the sensory neurones at various frequencies it can be shown that accommodation takes about 1/3 sec. to become fully established, stimulation during this period being effective in exciting the giant fibre.

It appears that rapid adaptation of the sensory endings in the integument does not occur since, long after giant fibre impulses have ceased to be produced, small slow impulses in the nerve cord can be elicited by mechanical stimulation of the body wall.

The existence of these junctions and pathways has not yet been confirmed histologically and this is a problem for future investigation.

I am indebted to Prof. C. F. A. Pantin and also to Dr. G. A. Horridge of the Gatty Marine Laboratory in which much of this work was done.

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Responsiveness of the Neurosecretory System of Larval Lampreys (*Petromyzon marinus*) to Light

IT now seems reasonable to conclude that the brain, and in particular the hypothalamus, mediates most endocrine changes, which are responses by vertebrates to changes in their physical environment. The terminal nervous pathway in this integrative system is the hypothalamo-hypophyseal tract which extends from the preoptic (supraoptic) and paraventricular nuclei to the neurohypophysis[1]. The neurones in these hypothalamic nuclei are secretory; the secretory material is stainable, and there is evidence that through special circulatory connexions it may be transported from the nerve endings to the glandular hypophysis to stimulate the release of the trophic hormones[1,2]. Through this neuroendocrine axis, therefore, the environmental regulation of reproduction, behaviour and metabolism of vertebrates may be expressed.

It seemed to us important from the phylogenetic point of view to learn whether such a hypothalamo-

hypophyseal neurosecretory system functions in the most primitive of living vertebrate forms. For this purpose we used ammocœtes (larvæ of the lamprey *Petromyzon marinus*), and tested the responsiveness of their hypothalamic neurosecretory apparatus to fluctuations in external illumination. It is well known that increases in external illumination have a strong stimulatory effect on gonadal function of various vertebrates[1], and recently, in amphibians, birds and rats, it has been shown that varying the environmental photoperiod produces changes in the hypothalamic neurosecretory system extensive enough to be visible even in histological preparations of the hypothalamus[2–6].

Thirty-eight ammocœtes of *Petromyzon marinus* measuring 2·0–10·5 cm. were obtained from the Salmon River near Plattsburg, New York, in October 1958. In March 1959 they were divided between two glass aquaria in aerated water kept at the same temperature (22° C.). One aquarium was completely covered with a black paper hood. The other was constantly illuminated by a 60-watt electric bulb, 5 in. above the aquarium. Five animals were removed from each aquarium at 1-, 2-, and 3-week intervals. The remaining eight animals at the end of the 3-week period were exposed to laboratory daylight (approximately 12-hr. days) for one week.

Serial sections 5–7μ thick were prepared of the heads after Bouin–trichloracetic acid fixation. Staining was by the Gomori hæmatoxylin–phyloxine technique, or aldehyde–fuchsin, or periodic acid–Schiff reactions[7–10].

The hypothalamic neurosecretory system of adult *Petromyzon marinus* has been described by others[11–14], and that of the ammocœtes, with minor differences, resembles it. The preoptic nucleus consists of uniformly sized bipolar cells (nuclear diameter 3·5–4·5μ). Aldehyde fuchsin and Gomori stainable granules are found in both the 'dendritic' process and long axon as well as in the cell body. In the dendrite granules appear to be moving toward, and released into, the third ventricle. Axons from the preoptic nuclei are gathered into two tracts which end in the neurohypophysis. Even in the smallest larvæ the neurohypophysis contains accumulated aldehyde–fuchsin and Gomori-stainable material, a fact which may be taken as evidence of early secretory activity in the preoptic nucleus. A second, more posterior, nucleus of neurosecretory cells is found at the recessus posterior close to the mesencephalon. These slightly larger bipolar cells (nuclear diameter 4·3–5·0μ) are also uniform in size. They, too, have a shorter dendritic fibre which appears to conduct granules to the adjacent ventricle. A minority of the axons of the posterior group terminate in the neurohypophysis; most terminate near capillaries in the connective tissue covering the anteroventral part of the mesencephalon.

External illumination clearly influenced the amount of stainable hypothalamic neurosecretory material that could be found. In larvæ kept in the dark there was a strong accumulation of aldehyde–fuchsin-stainable granules in the preoptic nucleus, and in the preoptico-hypophyseal tract. In the preoptic nucleus, even after one week, there was a clear distinction from animals kept under normal daylight conditions. Accumulation of granules in dark-adapted animals was so dense that cellular details were difficult to distinguish. The secretory neurones near the posterior recess, on the other hand, did not appear in any way altered.

Continuous illumination had the opposite effect. With time the cell-bodies and the axons were almost completely depleted of aldehyde–fuchsin- or Gomori-stainable granules. However, the neurohypophysis was still full of stainable material. By contrast, the cells near the recessus posterior were unaffected, even after three weeks. Returning the dark-adapted or continuously illuminated larvæ to normal daylight illumination reversed the extreme changes seen in each group.

Thus, it seems justifiable to conclude that the hypothalamus of the larval lamprey contains a functional neurosecretory system. Furthermore, it is differentiated into an anterodorsal pre-optic region the elaboration and release of neurosecretion of which is responsive to changes in external illumination, and a postero-ventral region which is unresponsive to this environmental factor. The presence of such a system in these primitive vertebrates indicates the early evolution of the hypothalamo-hypophyseal form of neuroendocrine control in vertebrates and may now permit comparisons with similar systems in the invertebrates. Further study will be needed to show whether the system in cyclostomes in the more detailed aspects of its function has properties which may be considered different from or more primitive than those which have been described in birds and mammals.

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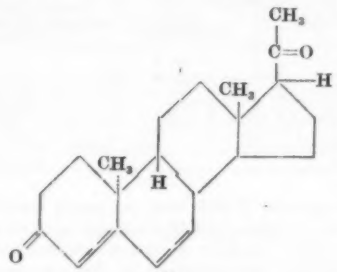
PHARMACOLOGY

A New Class of Hormonally Active Steroids

The belief that the naturally occurring steroid hormones represent the highest attainable biological activity has been disproved by recent work. Most emphasis has been placed on the introduction of substituents in the steroid nucleus, while changing the configuration at the centres of asymmetry has received relatively little consideration[1–3] and has so far not led to therapeutically active compounds.

In order to study the influence of stereochemical changes on the pharmacological properties of steroids, we prepared a number of steroid hormone analogues with the same configuration of the C-9-hydrogen

atom and C-10-methyl group as present in lumisterol$_2$. Recent investigations[4] showed lumisterol$_2$ to have a 9β,10α configuration. We wish to report on the pharmacological properties of some of the compounds prepared. 9β,10α-Progesterone(9β,10α-pregn-4-ene-3,20-dione) has marked progestational activity when administered orally or systemically to œstrone-primed immature rabbits. The potency of the subcutaneously administered compound is approximately five times that of progesterone. Other compounds in the 9β,10α-pregnane series such as 9β,10α-pregna-4,6-diene-3,20-dione (I) are even more active in this respect.



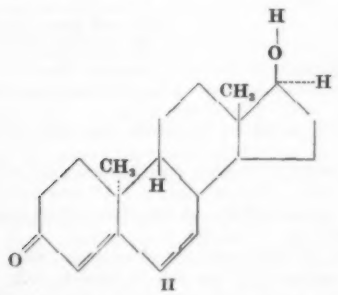
I

Subcutaneous administration of this compound to rabbits and comparison with progesterone in the McPhail[5] test indicate that this compound is approximately 25 times as active as progesterone. Also, oral administration in the same test showed these new compounds to be highly active.

No androgenic activity could be demonstrated in infantile and castrated rats at dose-levels up to 2 mgm. twice daily subcutaneously[6].

In clinical studies 9β,10α-pregna-4,6-diene-3,20-dione was administered to women with primary or secondary amenorrhea, who had been pretreated with ethinylœstradiol. A complete secretory change in the endometrium was achieved after oral administration of doses of 10–20 mgm. daily, during 10 days. All women reacted with withdrawal bleeding within 3 days. No side-effects were observed[7].

Other interesting compounds were found in the 9β,10α-androstane series. 9β,10α-Testosterone (17β-hydroxy-9β,10α-androst-4-en-3-one) possesses no androgenic activity, not even at dose-levels of 2 mgm. twice daily subcutaneously in infantile and castrated rats. 17β-Hydroxy-9β,10α-androsta-4,6-dien-3-one (II) was shown to possess a definite anti-androgenic activity[8].



II

These results seem to indicate that a stereochemical change at carbon atoms 9 and 10 will lead to steroids with interesting pharmacological properties.

Full reports of the chemistry[9], pharmacology[6,8] and clinical effects[7] of these steroids will shortly be published.

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Tolerance to Drugs, especially to Morphine

Various theories have been put forward to elucidate the development of tolerance to a drug. For example, it has been observed by Zauder[1] that "as tolerance develops (in the rat), the amount of morphine appearing in the urine decreases". This was in accordance with Gross and Thomson's observations on the dog[2]. Cochin *et al.*[3], on the other hand, could not elucidate any significant differences in the pattern of excretion and, on their own admission, were at a loss to "account for the development of tolerance and physical dependence in the dog", and suggested that "*it becomes imperative to look to other explanations for the mechanisms of development of these phenomena*".

Evidence has been found in this laboratory, in accordance with Beutner's suggestion[4], that morphine interacts with serum proteins. However, the possibility that such an interaction might be responsible for unusual resistance or tolerance to various drugs does not appear to have been systematically investigated to this date.

Human and dog sera, freshly collected and kept at 37° C., were ultracentrifuged (at 35,000 r.p.m. for 16 hr.) after the addition of small amounts of morphine (10 and 20 μgm. per ml.). Proper care was observed to keep the samples as close as possible to physiological conditions. The distribution of morphine paralleled grossly that of the proteins in the ultracentrifugates, and the phenomenon was much more marked with human than with dog serum. Quantitative estimation of morphine was performed according to a method previously described by us[5].

This actual demonstration of the binding properties of serum proteins towards morphine suggested an

investigation of the distribution of both free and protein-bound morphine in the various tissues and fluids of tolerant and non-tolerant dogs. The animals were made tolerant to morphine according to the procedure described by Cochin *et al.*[3]. Some evidence has indeed been found that the development of tolerance is accompanied with a decrease in the rate of disappearance of the drug from the blood following a single injection (5 or 10 mgm./kgm.). No morphine was detected in the cerebrospinal fluid of either group, 1 hr. after the injection. The distribution of morphine in the various fractions of blood ultracentrifugates obtained from both groups was in accordance with an increase in the binding properties of the serum of tolerant animals.

Although this work is still at a preliminary stage, we are inclined to agree with Goldstein's view that "the primary effect of (protein) interaction is . . . to confine the drug molecules within the blood stream in an inactive form, and thereby hinder their access to the sites of drug action, excretion and metabolism"[6].

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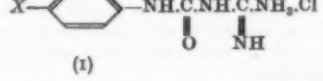
Antimalarial Properties of some Guanidine Derivatives

It has been shown by two of us[1] that many aromatic primary and aromatic–aliphatic secondary amines can react with cyanguanidine to yield N^1-aryl-N^3-amidine-ureas and N^1-aryl-N^1-alkyl-N^3-amidine-ureas respectively, under certain experimental conditions. (The reaction between the amines and cyanguanidine leading to arylbiguanides is already known.)

A number of aryl amidine-urea derivatives of the general formula (I) were investigated against chicken malaria by two of us (Y. Ch. Ch. and Y. Y. W.). Paludrine was also used as standard.



(I)

The experiments were carried out with chickens, five in a group, weighing 40–55 gm. each. Each was inoculated into the jugular vein with 25×10^6 *Plasmodium gallinaceum*. The substances under test were given *per os*, starting the same afternoon following the inoculation. In the following three days, one dose was given twice a day, in the morning and in the afternoon. Thus a total of seven doses was administered to each bird. A series of control chickens was infected but untreated.

Each dose consisted of about one-tenth of the respective *LD*50. On the fifth day, when the red corpuscles of the chickens in the control group were infected to the extent, on average, of 300 per thousand or more, blood smears were made from all experimental birds. Counts of normal and infected red corpuscles were made, following Giemsa staining. Two of the compounds examined, namely, *T* 72 (I, $X = NO_2$) and *T* 261 (I, $X = \overset{+}{N}H_3\overset{-}{Cl}$), have been found to be plasmodicidal.

The results of the preliminary experimental therapy are given in Table 1.

Table 1

Substance	Dose (mgm./kgm.)	Average number of infected red corpuscles (per thousand)
T 72	50	—
	100	—
T 261	50	11·6
	100	13·6
Paludrine	4	4·4
	8	—
Control	—	377

The compound *T* 261 was definitely active at 50 and 100 mgm./kgm., but the body-weight of chickens receiving these dosages decreased considerably.

More detailed investigation of the compound *T* 72 was carried out with smaller doses. The results are given in Table 2.

Table 2

Substance	Dose (mgm./kgm.)	Average number of infected red corpuscles (per thousand)
T 72	40	±
	20	31
	10	351
	5	290
	1	325
Paludrine	40	—
	20	—
	10	±
	5	0·8
	1	202
Control	—	397

T 72 was found to be one-quarter as potent as paludrine.

The acute toxicity was examined on mice and rats by introducing the substances by stomach tube. Each animal was given a single dose and deaths recorded until the third day. The results are shown in Table 3.

Table 3

Substance	*LD*50
T 72	1,200 mgm./kgm. (mice) 1,790 mgm./kgm. (rats)
T 261	*c.* 1,000 mgm./kgm. } (mice)
Paludrine	45 mgm./kgm. }

Thus *T* 72 is about 26 times less toxic than paludrine.

The subacute toxicity was investigated during six weeks in rats. The substance *T* 72 was administered daily by stomach tube at the dose of 90 mgm./kgm. (one-twentieth of *LD*50).

This did not produce any change in blood morphology of either red or white blood cells. Slight albuminuria appeared in some cases. No other pathological changes in urine were noticed.

The increase of body-weight was slightly less than in untreated animals. This may be attributed to the

irritation of the œsophagus by administration every day of the substance by the stomach tube.

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HÆMATOLOGY

An Rh Antibody Specific for V and R′ˢ

The serum of an Rh negative White woman, Mrs. V. S., contains an antibody which reacts only with the cells of people who have either of two predominantly Negro characters—V and R′ˢ (Table 1). The place of these antigens in the Rh system was previously unknown and a relationship between them unsuspected.

Table 1. Results of Testing the Red Cells of 262 Unrelated but often Selected People with the Serum of Mrs. V. S.

	Negroes				Whites			
	V	R′ˢ	R′	Others	V	R′ˢ	R′	Others
VS+	28	13	0	0	3	2	0	0
VS−	0	0	1	50	0	0	80	85*

* Includes one example of r^{G}r and representatives of almost all the known Rh genotypes.

The antigen V[1,2] has, up to now, resisted classification in terms of CDE, though a recent suggestion[3] was that V is the product of interaction between c and e in *cis* when one or the other of these genes is in an allelic form commoner in Negroes than in Whites.

For some years we and others have been aware that R′r (*dCe/dce*) is usually different in Negroes and Whites: the cells of the great majority of White R′r people are agglutinated by all anti-C sera while the cells of most Negro R′r (R′ˢr) people fail to react, or react weakly, with the anti-C sera of the type whose main antibody component is anti-Ce. The subject has been ably studied by Sturgeon, Fisk, Wintler and Chertock[4] and by Sturgeon[5], who give the symbol rh′ⁿ to what we are calling R′ˢ. (We have avoided n for negro because such racial labels have been objected to and because the character is not confined to Negroes.)

The antibody in the serum of Mrs. V. S., which by chance may conveniently be called anti-VS, clearly distinguishes an antigen common to V and to R′ˢ: absorption of the serum with cells containing V or R′ˢ greatly reduces the strength of the reactions for both kinds of cells. Furthermore, eluates from the absorbing cells confirm these results: eluates from cells containing V react with R′ˢ cells and vice versa.

To make a pattern for the reactions of anti-V and anti-VS all that is needed is the postulation of an antigen eˢ: V would correspond to antigens eˢ and ceˢ, and R′ˢ to eˢ and Ceˢ; anti-V would be anti-ceˢ and anti-VS would be anti-eˢ. The hypothesis would adumbrate the existence of an antibody anti-Ceˢ: it certainly explains neatly the negative or weak reactions of R′ˢr (*dCeˢ/dce*) cells with anti-Ce sera. Family tests would be needed to demonstrate *DCeˢ*—if it exists.

Growing appreciation of the complexity of Rh prevents us being very confident of any labelling of genes and antigens. However, it is now clear that the antigens V and R′ˢ no longer present particular Rh problems: their behaviour is merely reflecting that of other antigens controlled by *CE* gene complexes.

We are particularly indebted to Miss Lillian Teeters, chief blood bank technologist, working under the supervision of Dr. H. A. van Auken, pathologist, of the Baptist Memorial Hospital, San Antonio, Texas, who first realized that the serum of Mrs. V. S. deserved elaborate investigation. Acknowledgment to many others who have helped will be made in the detailed account which will be submitted for publication elsewhere.

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Micro-electrophoresis of Human White Cells and Platelets

We have previously reported our experience with determining charges on human red cells, and the changes in charge which occur when the cells are treated with appropriate antisera[1,2]. The purpose of this communication is to present analogous results for human white cells and platelets.

Blood from normal human donors was received into disodium versenate or sodium citrate, and white cells recovered either by centrifugation and buffy coat withdrawal, or by a sedimentation method using polyvinyl pyrollidone. Platelets were obtained by bleeding normal human donors into siliconized vessels containing disodium versenate or sodium citrate. The preparation was then centrifuged at 1,000 r.p.m. for 10 min., the supernatant removed, and again centrifuged at 3,000 r.p.m. for 10 min. Residual red cells were lysed with 1 per cent acetic acid, and the platelets washed three times in phosphate buffer[3].

Table 1

		No. of left–right chamber runs	Mean migration time (sec.)*	No. of right–left chamber runs	Mean migration time (sec.)*	Mean migration value (μ) × 10^{-4} cm.^{2}/V. sec.
With unsensitized white blood cells from subject of blood group	A, D pos.	10	12·08 (+0·32, −0·48)	10	12·4 (+1·2, −0·8)	1·28
	A, D pos.	10	12·4 (+0·4, −0·4)	10	12·42 (+1·18, −0·82)	1·27
	B, D pos.	10	13·5 (+0·55, −0·25)	10	13·05 (+0·15, −0·05)	1·21
	B, D pos.	10	12·48 (+0·32, −0·88)	10	12·36 (+0·44, −0·76)	1·27
	O, D pos.	10	12·46 (+0·34, −0·26)	10	12·40 (+1·2, −1·6)	1·27
	O, D neg.	10	12·52 (+1·48, −0·52)	10	12·36 (+0·64, −0·36)	1·26
	O, D neg.	10	12·44 (+0·36, −0·44)	10	12·52 (+0·28, −0·52)	1·26
With sensitized white blood cells from subject of blood group	O, D pos.	10	13·46 (+0·14, −0·26)	10	13·42 (+0·18, −0·20)	1·16
	O, D pos.	10	13·44 (+0·56, −0·64)	10	13·44 (+0·56, −0·64)	1·16
	O, D neg.	10	13·72 (+0·28, −0·12)	10	13·92 (+0·48, −0·32)	1·14
With unsensitized platelets from subject of blood group	O, D pos.	10	2·92 (+0·28, −0·18)	10	2·97 (+0·53, −0·17)	5·3
	O, D pos.	10	2·90 (+0·30, −0·30)	10	2·74 (+0·26, −0·34)	5·5
	O, D pos.	10	2·96 (+1·04, −0·56)	10	2·57 (+0·43, −0·37)	5·6
	O, D neg.	10	3·28 (+0·52, −0·28)	10	3·18 (+0·62, −0·38)	5·09
	O, D neg.	10	2·70 (+0·30, −0·30)	10	2·55 (+0·45, −0·55)	5·8
	B, D pos.	10	3·02 (+0·18, −0·42)	10	2·70 (+0·30, −0·30)	5·4

* The migration time of each of 10 cells is determined in the left–right chamber direction, and the migration of 10 additional cells is determined for the right–left chamber direction. The mean migration time is recorded and the bracketed figures indicate the extremes of behaviour above and below the mean value.

When white blood cells were to be treated with antisera, 5 c.c. of polyvinyl pyrollidone was added to 20 c.c. of defibrinated normal human blood. After incubation for 1 hr. at 37° C., the supernatant was removed and centrifuged, the resulting button of cells being then treated with 1 per cent acetic acid to lyse the residual red blood cells; 2 c.c. of leucoagglutinating serum (titre 1 : 32), kindly supplied by Dr. Rose Payne, Department of Medicine, Stanford University School of Medicine, Palo Alto, California, was added to the button of white blood cells and incubated for 1 hr. The white blood cells were then washed three times in phosphate buffer and considered as 'sensitized' white blood cells.

Suitable suspensions of white blood cells or platelets in phosphate buffer were then submitted to electrophoresis in a cataphoretic cell as previously described[1,2].

The migration for white cells and platelets is shown in Table 1. As we found no differences in migration values whether disodium versenate or sodium citrate was used as the calcium binder, and no differences whether the buffy coat or polyvinyl pyrollidone method of separating white cells was employed, separate results are not given for these alternative treatments.

The observations indicate that human white cells are negatively charged, but slightly less so than red cells, which possess migration values of 1·31 or greater[1,2]. Leucoagglutinin treatment of these white cells produces a change of charge as with red cells, but the change is in the direction of less negativity in contrast to the increased negative charge seen with the action of anti-red cell antibodies on red cells[1,2]. The same diminution in negative charge of white blood cells treated with antibody has recently been reported using guinea pig white cells and rabbit antisera[3]. In addition, the magnitude of the change of charge on white blood cells following leucoagglutinin treatment was about half that found after treatment of red cells with their antibodies. (The assertion that sensitized white cells give migration values lower than unsensitized ones is supported by the results in Table 1 at the 1/165 level of significance, that is, there is one chance in 165 that a group of 4 and a group of 7, constituting a homogeneous lot of 11, should have no overlap merely by accident.) The charge of sensitized or unsensitized white cells appeared to be unrelated to red cell group in so far as determined, and this again forms a contrast to the micro-electrophoretic behaviour of red cells[1].

We have had only limited experience with platelets, and none with the possible effect of anti-platelet antisera on normal platelet charge. The platelets of six subjects have been studied, and all were found to possess very high negative charges, approximately four times those of red cells or white cells. The strong negative charge of platelets may be of importance in explaining the frequency with which these formed elements of the blood are involved in adverse reactions to drugs.

We are continuing this work on the normal micro-electrophoretic characteristics of the formed elements of the blood and the effect of relevant antibodies on them with the view of supplementing information from serological identification of antibodies related to the cells of the blood. Further, the technique used may prove suitable for *in vitro* prediction of success for therapeutic agents proposed as capable *in vivo* of interfering with cell-antibody unions.

This work was carried out while one of us (P. M. T.) was holding the Beal Fellowship in Hæmatology at Stanford University School of Medicine.

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Influence of Dietary Intake of Certain Insecticides on the Hæmoglobin and Erythrocyte Contents of Chick Blood

Several of the modern synthetic organic insecticides are showing promise for control of the external parasites of poultry. It is now well known that these insecticides exert influence on the health of animals in varying degrees. Pigeons exposed to high con-

Table 1

S. No.	Treatment: Supplement	Treatment: Level p.p.m.	Age of chicks (days): 12 Hæmoglobin (per cent)	12 Red blood count (× 10^6 c.mm.)	17 Hæmoglobin (per cent)	17 Red blood count (× 10^6 c.mm.)	22 Hæmoglobin (per cent)	22 Red blood count (× 10^6 c.mm.)	32 Hæmoglobin (per cent)	32 Red blood count (× 10^6 c.mm.)
1	None	—	10·0	2·18	9·4	1·98	8·9	1·92	9·2	1·98
2	BHC	25	8·4	1·60	6·6	0·94	6·3	1·00	5·0	0·88
3	Dieldrin	1·25	7·7	1·70	6·5	1·01	6·3	0·98	5·5	0·95
4	DDT	35	7·7	1·65	6·5	1·08	6·2	1·04	4·8	0·90
5	Malathion	40	7·7	1·62	6·9	1·21	6·1	1·07	8·1	1·92

centrations of phosgene and mustard gases showed a lower hæmoglobin content of blood[1]. Oral administration of DDT to certain laboratory animals resulted in anæmia and leucocytosis[2]. So far as we are aware, no information exists on the toxicological effects of these insecticides on the blood picture of poultry. The present communication outlines the results of our experiments with chicks.

Twenty-five day-old chicks of the Rhode Island Red breed were randomly distributed into 5 lots of 5 chicks each. They were raised in electrically heated brooders developed at this College and fed a practical type of basal ration generally used on the State poultry farms. Feed and water were supplied *ad libitum*. After a period of 7 days, four insecticides at levels shown in Table 1 were incorporated in the diet. One lot received the basal diet to serve as a positive control. For recording the hæmatological observations chicks were bled from the brachial vein every fifth day from the date they were put on experimental diet. Three such observations were recorded, after which the chicks were again put on basal diet. Hæmoglobin and erythrocyte determinations were further made after a period of ten days. Hæmoglobin was determined on the Hellige scale by means of a hæmometer using 0·1 *N* hydrochloric acid, and erythrocyte counts were made by means of a standard Fien-Optic hæmocytometer having improved double neubauer rulings.

The results are presented in Table 1. It is evident that feeding insecticides to chicks resulted in a significantly lower hæmoglobin and erythrocyte content of blood in all cases, which was noticeable even on the fifth day after chicks were fed insecticides. The values for both the constituents further decreased progressively in subsequent observations. The observations taken 10 days after chicks were fed basal diet again showed a further decrease in hæmoglobin and erythrocyte contents of the blood from chicks that received DDT, BHC and dieldrin, while the chicks that had received malathion, an organic phosphate, had returned to normal levels of both these constituents. The chicks that received chlorinated hydrocarbons (DDT, BHC and dieldrin) showed a hock disorder similar to perosis which gradually resulted in loss of weight and 50 per cent mortality of chicks at the age of 32 days.

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Initiation of Clotting in Normal and Hageman Plasma by Glass

Plasma from a patient with the Hageman trait cannot be activated by contact with glass[1,2]. Bird plasma behaves as Hageman plasma[1]. Hageman plasma is less able to correct defects in plasma samples from patients with various other hæmorrhagic diatheses than are other normal or deficient plasma samples[3]. While centrifugation cannot completely remove platelet activity in human plasma[4], this is possible in bird plasma. The possibility exists that the difference in clotting activity after activation of normal human plasma by glass on one hand and of normal bird plasma and Hageman plasma on the other is related to platelet activity.

Therefore, the recalcification times of platelet-free Hageman plasma (kindly provided by Dr. O. D. Ratnoff to Dr. Tage Astrup) and of platelet-free normal human plasma were compared with those obtained after addition of a strong platelet suspension. The following results were obtained:

	Hageman plasma	Normal plasma	Hageman plasma + platelets	Normal plasma + platelets
Recalcification time (sec.)	910	242	90	61

The experiment shows that after addition of excessive platelets Hageman plasma clots almost as rapidly as normal plasma. This observation could explain why Sjølin[5] was able to correct the defect in a sample of plasma from a person with an apparent Hageman trait by addition of the person's own platelets.

In a further experiment platelets were added to Hageman plasma which had first been exposed to glass. The recalcification time was 252 sec. When the platelets were added to the plasma before its exposure to glass the recalcification time was 95 sec. This shows that it is possible to activate Hageman plasma by glass when sufficient platelet activity is present during the activation.

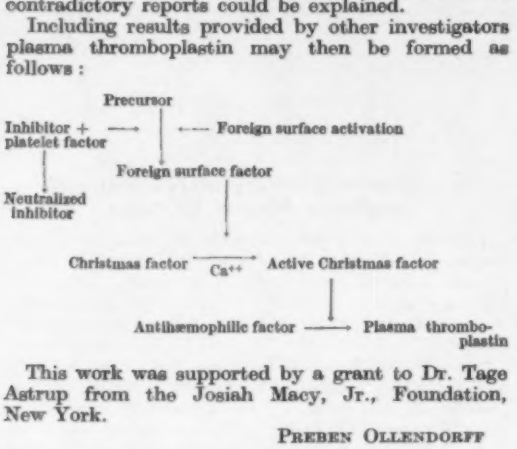
I have also found samples of normal, platelet-poor plasma which could not be activated by glass, whereas the same platelet-rich plasma was easily activated. Apparently in normal plasma the presence of platelets, or of platelet activity, is essential for the activation by glass.

These experiments suggest that coagulation in glass tubes could be initiated by formation of a 'foreign surface factor' by activation of a precursor in plasma, and furthermore that activation of the precursor to 'foreign surface factor' is counteracted by an inhibitor which can be neutralized by a platelet factor, thus explaining why platelets are essential for activation by glass.

'Hageman factor' as well as 'plasma thromboplastin antecedent' ('PTA' or Rosenthals factor) participates in activation by glass[6,7]. If it is assumed that the

Hageman trait is not caused by a deficiency of a clotting factor, but by the presence of an excess of an inhibitor against the activation of 'foreign surface factor', and, moreover, that the factor is identical with 'plasma thromboplastin antecedent', many contradictory reports could be explained.

Including results provided by other investigators plasma thromboplastin may then be formed as follows:



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PATHOLOGY

Effect of 3-Amino-1,2,4-triazole on the Experimental Production of Liver Cancer

It is well known that the activity of liver catalase is definitely reduced in the course of 4-dimethylaminoazobenzene carcinogenesis in rat liver[1]. Nakahara and Fukuoka[2] reported that this reduction of liver catalase was prevented if liver powder or blood meal was fed so as to inhibit the production of liver cancer and suggested that liver catalase played a significant part in the biochemical mechanism of carcinogenesis and anticarcinogenesis. Heim *et al.*[3], in 1955, first observed that intraperitoneal injection of 3-amino-1,2,4-triazole remarkably reduced the catalase activity of the liver and the kidney, not of the erythrocyte. It has been shown since then that 3-amino-1,2,4-triazole has a highly specific effect on liver and kidney catalase. It may therefore be interesting to study the effect of a prolonged low level of liver catalase activity by the use of 3-amino-1,2,4-triazole on the experimental production of liver cancer by azo dye. Based on the above views, the following experiments were performed.

The animals used in these experiments were male albino rats of mixed breed weighing 90–110 gm., two or three months old. The basal diet consisted of unpolished rice, supplemented by green vegetables every two days and water *ad libitum*. 4-dimethylaminoazobenzene was incorporated into the basal diet at a concentration of 0·06 per cent.

First, in order to confirm that 3-amino-1,2,4-triazole was able to inhibit the liver catalase activity even in the rats fed dimethylaminoazobenzene and with repeated injections of 3-amino-1,2,4-triazole, so that a prolonged low level of liver catalase could be maintained, the following experiment was carried out. Sixty rats were divided into two main groups: one was a dimethylaminoazobenzene diet group, the other a basal diet group. Both the groups were divided further into three groups: the first group was not treated with 3-amino-1,2,4-triazole, the second was injected intraperitoneally with 3-amino-1,2,4-triazole once 3 hr. before being killed and the third was injected with 3-amino-1,2,4-triazole every two days; in the last group the final injection was done 3 hr. before being killed. 3-amino-1,2,4-triazole was dissolved in cold sterile distilled water at a concentration of 10 per cent, and injected into rats in a dose of 1,000 mgm./kgm. On the thirtieth day after commencement of the experiment, all the rats were killed and the catalase activities of the livers titrated by the method of Euler and Josephson[4]. The nitrogen content of the liver homogenates was measured by the indophenol colorimetric method by Lubochinsky and Zalta[5] and catalase-levels were finally expressed in mean *Ko*/mgm. nitrogen.

As shown in Table 1, the result of this experiment indicated that dimethylaminoazobenzene feeding did not disturb the 3-amino-1,2,4-triazole action on the liver catalase, and rather somewhat accelerated its action, and repeated injections of 3-amino-1,2,4-triazole were able to maintain a prolonged low level of liver catalase activity.

Table 1. Liver Catalase Activity on the Thirtieth Day

		No. of rats	Mean *Ko*/mgm. N ± *S.D.*	Percentage
A	(1) Basal diet only	10	$(43{\cdot}01 \pm 8{\cdot}01) \times 10^{-3}$	100
	(2) Basal diet + *AT* once	10	$(4{\cdot}11 \pm 0{\cdot}43) \times 10^{-3}$	9·5
	(3) Basal diet + *AT* 14 times	10	$(3{\cdot}32 \pm 0{\cdot}51) \times 10^{-3}$	7·7
B	(4) *DAB* diet only	10	$(32{\cdot}82 \pm 1{\cdot}64) \times 10^{-3}$	76·1
	(5) *DAB* diet + *AT* once	8	$(2{\cdot}84 \pm 0{\cdot}29) \times 10^{-3}$	6·6
	(6) *DAB* diet + *AT* 14 times	8	$(2{\cdot}04 \pm 0{\cdot}36) \times 10^{-3}$	4·7

AT, 3-amino-1,2,4-triazole; *DAB*, dimethylaminoazobenzene.

Then the effect of 3-amino-1,2,4-triazole on the dimethylaminoazobenzene carcinogenesis was studied in the following experiment. Sixty rats fed dimethylaminoazobenzene diet were divided into two groups; one group was injected with 3-amino-1,2,4-triazole every two days in the same manner as in the former experiment; the other, a control group, was injected with an equal volume of sterile distilled water. Both injections were begun simultaneously at commencement of dimethylaminoazobenzene feeding, and continued until the animals died or were killed. The amount of dimethylaminoazobenzene ingested by each rat of both groups reached about 540–630 mgm. for five months. During the course of this experiment, 14 out of 30 in the control group and 11 out of 30 in the 3-amino-1,2,4-triazole-treated group died of respiratory infections or intoxications of the azo dye. Those which died within 90 days showed no neoplastic changes anywhere in the livers of either group and were omitted from the results.

All the rats which survived were killed on the one hundred and fiftieth day. In the treated group, the average body-weight amounted to 128·5 gm. and average liver weight 6·6 gm.; in the control group, 116·5 gm. and 7·5 gm. respectively.

The incidence of liver tumours in rats was 4 out of 19 in the treated group against 12 out of 16 in the control group. The difference which existed between both groups is statistically significant ($pr\{\chi^2 > 8{\cdot}128\} < 0{\cdot}01$), indicating that the treatment of 3-amino-1,2,4-triazole delayed the production of liver cancer by dimethylaminoazobenzene.

The livers of 3-amino-1,2,4-triazole-treated rats showed almost normal appearance and few cases were cirrhotic. Metastases could not be detected in any cases, whereas in the control animals tumour growth and cirrhosis were marked and metastases were also recognized in the regional lymph nodes.

Histologically, the hepatic carcinomas produced in the control rats were, for the most part, hepatocellular carcinomas, and those in the 3-amino-1,2,4-triazole-treated rats were intermingled with both hepatocellular carcinomas and papillary cystoadenocarcinomas (cholangiocarcinoma). In addition, precancerous lesions (cholangiofibrosis and nodular hyperplasia of the parenchymatous cells) and cirrhosis prevailed in almost all cases of the control group. On the other hand, these changes were found in few cases of the treated rats which bore no tumours.

I expected from the reports of previous workers[1,2] that, with repeated injections of 3-amino-1,2,4-triazole, dimethylaminoazobenzene carcinogenesis might be accelerated. However, contrary to my expectations, the results obtained show that 2-amino-1,2,4-triazole delays dimethylaminoazobenzene carcinogenesis. At present I have not sufficient data to explain this fact, but it may be conceivable that the delay of dimethylaminoazobenzene carcinogenesis is due to the other unknown action of 3-amino-1,2,4-triazole. Longer experiments with more experimental animals are under way with the view of obtaining definite conclusions.

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Loss of Specificity on Passage of Immunologically Competent Cells in the Chick Embryo

WHEN leucocytes from the blood of a fowl more than a few weeks old are placed on the chorioallantois of standard 12-day chick embryos, focal proliferative lesions are produced. These are well developed at four days and by seven days have become nodules 2–4 mm. in diameter[1]. Approximately 2×10^4 leucocytes produce one lesion. Owing to the occurrence of secondary foci, there may be considerable uncertainty about the count with some membranes, but when dilutions are used the distribution of counts obtained is close to a standard Poisson distribution.

Table 1. ACTION OF LEUCOCYTES FROM PURE LINE FOWLS ON ISOLOGOUS AND HOMOLOGOUS CHICK EMBRYOS

Leucocyte donor	Group *A* embryos	Group *B* embryos	Random embryos
A 3928 ♀	20 : 2 : 14	—	14 : 0 : 1
3763 ♀	2 : 1 : 5	—	—
4318 ♂ (sire)	1 : 4 : 11	4 : 0 : 6	16 : 0 : 0
B 6557 ♀	26 : 3 : 1	2 : 1 : 23	4 : 0 : 0

Table 2. ACTION OF CELLS FROM FIRST PASSAGE EMBRYONIC SPLEENS ON ISOLOGOUS AND HOMOLOGOUS CHICK EMBRYOS

Original donor	Group *A* embryos	Group *B* embryos
A 3763	8 : 2 : 0	4 : 0 : 0
4318	20 : 1 : 0	6 : 1 : 1
B 6557	21 : 0 : 0	21 : 3 : 6

In both tables the grouped figures represent in sequence: number of embryos showing standard count of foci-number, with foci but less than 10 per cent of standard-number, with no foci.

Quite different results are obtained when both leucocyte donor and recipient embryos are obtained from the same pure line stock. In these circumstances a high proportion of embryos show no lesions[3].

Through the co-operation of Dr. J. A. Morris, of the Commonwealth Scientific and Industrial Research Organization Poultry Research Centre, Werribee, we have been able to use two lines *A* and *B* which have been established by close inbreeding since 1948. They are derivatives of the White Leghorn stock *B*1*A* originally selected for high egg-laying capacity. As is common experience with highly inbred poultry lines, the eggs showed relatively low fertility, particularly in group *B*, and it has not yet been possible to carry out all experiments on the scale that would be desirable.

Table 1 shows the results obtained by testing leucocytes from three group *A* and one group *B* donors on isologous embryos and on either the other pure line or random embryos. In addition to embryos showing approximately the full number of foci, one finds some membranes with a small number of definite foci (< 10 per cent of the mean standard number) and others completely free from foci. In Tables 1 and 2 the numbers of embryos in these groups are shown in the form 20 : 2 : 14. In this set 20 embryos showed counts within the normal range, 16 having greatly reduced or zero counts. In all experiments the standard mean count was in the range 25–120 foci. One donor, 3928 *A*, gave close to 50 per cent of reduced or zero counts on isologous embryos, the other three a much higher proportion. It is of interest, however, that some normal high-count membranes were obtained in all groups.

When embryos are inoculated with leucocytes (not isologous) by either chorioallantoic or intravenous routes, nodular lesions develop in the spleen and less regularly in other organs. A cell suspension from such a spleen when inoculated on the chorioallantois produces proliferative foci of the same type as those obtained from adult leucocytes. Since normal embryonic spleens have no action whatever, the simplest interpretation is that the cells responsible are descendants of those primarily inoculated. The specificity of their activity was tested by inoculation on to embryos of groups *A* and *B*, with the results shown in Table 2.

Most passages were made through random embryos, but in two of the experiments with leucocytes 4318 *A* and 6557 *B*, passage was through the opposite

pure line. Irrespective of the type of embryo used for passage all experiments showed sharp, almost complete, loss of specificity.

In contrast to the findings of Simonsen[3], we have found it impossible to maintain the pathogenic action for more than 3–5 transfers. We believe that the loss of specificity observed in the first passage may represent the first step in a series of changes by which the initially competent cell clones lose their capacity to produce a 'graft versus host' reaction.

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[2] Cock, A. G., and Simonsen, M., *Immunol.*, **2**, 103 (1958).
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Viability and Tetrazolium Salt in Ascites Tumours

The use of tetrazolium salts in experimental and human pathology is valuable if the materials being examined are vital organs or tissues, for degenerating or dead tissue loses the ability to reduce tetrazolium salts[1]. The formation of formazan is widely used for the determination of general metabolism[2]. Since this enzyme reaction can only occur in the living cell, and as there are rather few methods for determining viability, the formation of formazan was investigated in ascites tumour cells during the process of degeneration *in vitro*.

It has been shown in a previous investigation[2] that Ehrlich ascites tumour cells readily produce formazan if glucose is the substrate. In this case the most effective electron-acceptor is tetrazolium chloride. The investigation was carried out on Ehrlich/*ELT* Stockholm ascites tumour cells (by courtesy of Prof. G. Klein). The cells were taken from mice which had been inoculated 10 days previously. To prepare material for the investigation of the change in the enzyme activity which occurs *in vitro*, ten to fifteen million of the cells were suspended in a medium of the following composition: 5 ml. horse serum, 2 ml. chicken embryo extract, 3 ml. Tyrode solution, 50 mgm. glucose, 1,000 I.U. penicillin, 50γ streptomycin. This was incubated at 37° C. under sterile conditions.

The determination was performed as follows: the cell-suspension was centrifuged at 1,500 r.p.m. for 3 min. at certain fixed times. The sediment was then suspended in a medium which contained 1 ml. Ringer's solution, 1 ml. 1 per cent 2,3,5-triphenyl tetrazolium chloride, 0·5 ml. phosphate buffer (0·2 *M*, *p*H 8·04), 0·5 ml. glucose (0·01 *M*, the *p*H adjusted to 7·4); incubation 90 min., at 37·5° C. (± 0·5° C.), extraction with acetone. The absorption was measured with a Beckman spectrophotometer model *DU* at 484 mμ. Double determinations were made in every case. The dead cells were counted by Schrek's method[4]. At different phases of the *in vitro* 'tissue cultures' of ascites tumour cells morphological examinations of smears, stained with methyl green–pyronine after previous fixing in Carnoy, were made.

During *in vitro* conditions, when the cells were suspended in the above-mentioned medium, a gradually progressive degeneration could be observed in the phase-contrast microscope and, parallel to this degeneration, the cells lost their ability to grow after transplantation. The degeneration was particularly well observed on the smears stained with methyl green–pyronine. It was found that the formation of formazan decreased on degeneration. The production of formazan at 0 hr. varies considerably from experiment to experiment. In three parallel experiments, at 0 hr., values of $2{\cdot}2 \times 10^{-7}$ μgm., $2{\cdot}8 \times 10^{-7}$ μgm. and $3{\cdot}8 \times 10^{-7}$ μgm. of reduced tetrazolium chloride/cell/90 min. were measured. The value at explantation was taken as 100 per cent (0 hr.). After 24 hr. it is 40–50 per cent, 48 hr. 28–32 per cent, 72 hr. 15–18 per cent and after 96 hr. 7–9 per cent (Fig. 1). The dead cell count (using Schrek's method) showed very clearly that an increase in the number of dead cells led to a decrease in formazan formation. At explantation 3–6 per cent of all cells are stained. The number of stained cells, that is, dead cells, at 24 hr. is 68–74 per cent, at 48 hr. 88–93 per cent and 96 hr. as much as 96–98 per cent.

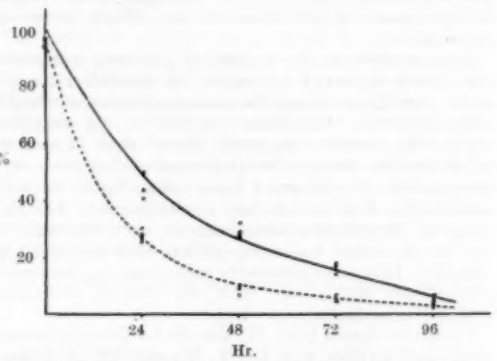

Fig. 1. Curves, ———, relative amount of formazan at different times (0 hr. = 100 per cent); – – – –, number of uncoloured cells per 100 counted (Schrek). Double determination throughout. The curves are the result of three experiments

This shows that ascites tumour cells *in vitro* lose the ability to reduce tetrazolium chloride. From the results of the experiments made one can say that a decrease in reduction of tetrazolium salt is a sign of the beginning of 'physiological' degeneration. However, it ought to be pointed out that probably not all factors hindering proliferation also cause a decrease in the reduction ability of tetrazolium chloride at the same time. If the hindering factors, directly or indirectly, cause the death of cells, then it is conceivable that this occurs parallel to a decrease in formazan formation. In this way the reduction of tetrazolium chloride can be used in tissue cultures, for example, in order to show the relation between living and dead cells clearly, presupposing that the living cells have a well-measurable enzyme activity.

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Effect of S-β-aminoethyl*iso*thiouronium Bromide Hydrobromide on X-ray Damage to Guinea Pig Skin

MANY chemical substances have been investigated for their ability to protect animals against the lethal effects of total-body radiation[1]. Of these, S-β-aminoethyl*iso*thiouronium bromide hydrobromide is one of the most effective if administered before exposure. It produces a remarkable degree of protection to total-body radiation in mice[2]. Similar protection is afforded to the monkey if given 150–250 mgm./kgm. prior to administration of 650 r. whole-body X-radiation[3]. The following experiments were carried out to test whether S-β-aminoethyl*iso*thiouronium bromide was capable of protecting tissues against local irradiation.

17 male albino guinea pigs of average weight 693 gm. (range 530–920 gm.) were given 5,000 r. to a 1-in. circular area of skin under 'Nembutal' anæsthesia. This dosage was one which caused deep ulceration in all normal guinea pigs and the details of administration have been previously reported (see ref. 4 and Mellett, P. G., Walter, J. B., and Houghton, L. E., unpublished work).

Seven animals were kept as control, the remaining ten being treated with S-β-aminoethyl*iso*thiouronium bromide. 100 mgm./kgm. were given the day before and 150 mgm./kgm. 10 min. before exposure. The experiment was repeated using nine control animals and ten treated. Their average weight was 472 gm. (range 420–500 gm.).

There was no significant difference between control and animals treated with S-β-aminoethyl*iso*thiouronium bromide, either in the development of the initial superficial ulceration or the later deep lesion. The results obtained in the second experiment are shown graphically in Fig. 1. Although 5,000 r. is a heavy dose of X-rays, smaller doses are insufficient to cause ulceration in 100 per cent of animals. Any substance of useful protective value should therefore be capable of detection using this dosage.

Much of the work on chemical protection against the effects of X-radiation has been done using lethal total-body exposure. It is evident that results obtained by this method are not necessarily applicable to those obtained using local heavy irradiation such as are used in clinical radiotherapy.

In another series of experiments, locally applied X-rays in doses up to 1,500 r. have delayed the contraction of circular excised wounds in rats. This effect can be antagonized by S-β-aminoethyl-*iso*thiouronium bromide given before the irradiation; thus this substance administered parenterally is capable of reversing this particular X-ray effect. However, it does not produce significant protection against ulceration caused by larger doses. It may be that the mechanism involved in the skin necrosis caused by irradiation is not entirely identical with the mechanism involved in either the action of X-rays on wound contraction or in the lethal effect of total-body irradiation in animals.

We wish to thank Mr. R. D. Chambers for technical assistance and Mr. D. E. A. Jones for his help with the irradiation.

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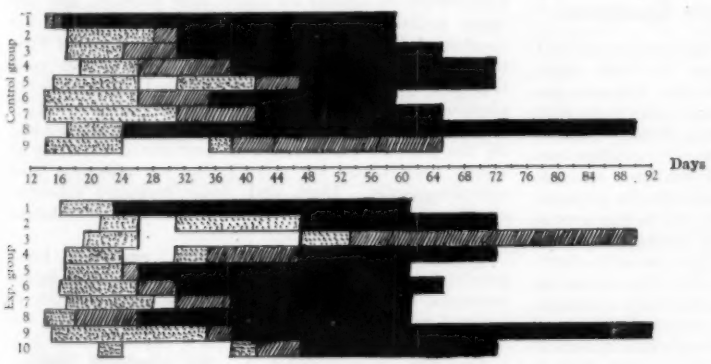

Fig. 1. Solid, large deep ulcer; hatched, small deep ulcer; stippled, superficial ulceration

BIOLOGY

Influence of Environment on the Distribution and Form of Sponges

DURING a taxonomic study of sponges in the Madras harbour and the adjoining Royapuram beach[1], certain differences in distribution, size, texture and the extent of animal associations between the sponges occurring in these two areas were observed.

The Madras harbour is a protected area with placid waters. Owing to the effluents from the ships and docks the water there is somewhat polluted. The side walls of the harbour and some boats docked there for long periods are the substrata on which the sponges occur. In addition, the dead bivalve shells on the side walls of the harbour also provide a good substratum for certain sponges. The Royapuram beach, on the other hand, is an open, sand and boulder beach, subject to heavy wave action.

Of the ten species studied, five (*Reniera delicatula, Mycale mytilorium, M. madraspatana, M. aegagropila* var. *militaris*, and *Callyspongia diffusa*) occur only in the harbour, while three (*Prostylissa arcotti, Tedania nigrescens* and *Pachychalina multiformis* var. *manaarensis*) were found only on the boulders of the Royapuram beach. Two forms (*Oceanapia arenosa* and *Lissodendoryx similis*) occur in both the localities. The harbour contains an abundantly larger sponge fauna than the Royapuram beach does.

A characteristic feature of the harbour sponges is their association with several groups of

animals. Sessile hydrozoans, polyzoans and sea anemones are attached to the surface while serpulids and sabellids live in tubes of their own buried within the tissues of the sponge with only their outer ends exposed. Asteroids, brittle stars, copepods, isopods and amphipods are seen to live in the canal systems of the sponges. Such animal associations are unusual in the case of the Royapuram sponges. Occasionally, however, a few colonies of hydrozoans or polyzoans are found growing on the surface of the sponges.

The harbour sponges are very fragile and soft, and easily torn, whereas the Royapuram sponges are firm in texture and are never soft. The harbour sponges are not as large as the Royapuram ones which are usually more than 8–10 cm. in length, 5–9 cm. in breadth and 3–5 cm. in thickness. No sponge collected in the harbour measured more than 5–6 cm. in length, 3–4 cm. in breadth and 1 cm. in thickness.

The harbour sponges vary in their mode of attachment to the substratum. Those that occur on the side walls and on boat-sides have a small base that serves as attachment to the substratum, and the sponge is flabby and branching. The sponges that occur on living and dead bivalve shells which in turn are attached to the side walls are found encrusting the shell surfaces. The Royapuram sponges are usually flatter and broader, with their entire base attached to the rocky substratum, and are also found to contain varying quantities of sand particles in their bodies.

The above-mentioned differences between the sponges occurring in these two habitats are clearly seen in *Oceanapia arenosa* and *Lissodendoryx similis*, which occur in both localities. The individuals collected from the harbour are different from those belonging to the same species but occurring on the Royapuram beach. The differences relate to the features enumerated above.

The development of the stereogastrula larvæ of *Lissodendoryx similis* that occurs in both localities was studied[2] and was found to be similar.

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The numbers in the column 'Months' give the number of months during which that species was observed, and, in parenthesis, the total number of months which include the first and last observations of it.

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More details will be published elsewhere, including observations on individuals of other species, and on imagines which have emerged in captivity.

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Fred, Baldwin and McCoy[1] made the generalization that effective strains of *Rhizobium* produce few but large nodules, usually located on the upper root system of the host, whereas ineffective strains form numerous small nodules widely distributed over the root system. Subsequent detailed studies on the physiology of nodulation have been summarized by Nutman[2], with a hypothesis relating nodule density on the root to the number of preformed foci, themselves determined by genetic factors affecting root morphogenesis. Dart and Pate[3] recently reported studies concerning the effects of delayed inoculation on nodule production and position.

Our observations with lupins, reported here, establish the importance of species in nodulation patterns, and suggest that it may be unwise to generalize from limited legume representation to 'legumes'.

Lupinus angustifolius L., *L. digitatus* Forsk., *L. luteus* L. and *L. mutabilis* Sweet were nodulated

by each of nine *Rhizobium* strains known to be effective in symbiosis with all species, in an experiment of factorial design: species (4) × strains (9) × replications (3). Plants were grown under controlled conditions in glasshouse sand-culture units. Four disinfected seeds were planted in each pot and inocula were applied to seeds in uniform quantities. Root systems were inspected after a period of plant growth of 50 days.

Three distinctive attributes of nodulation pattern were observed. Crown nodulation (*C*) was distinguished as any nodulation located on the tap root above the first lateral root. Tap-root nodulation (*T*) was distinguished as any nodulation on the tap root anywhere below the first lateral root. Lateral root nodulation (*L*) was distinguished as incidence of any nodules on lateral roots. Each attribute was not always represented in each replication of four plants. The frequencies of attribute incidence on the four species are given in Fig. 1, where the highest possible frequency is 27 (on the basis of 27 culture-unit replications).

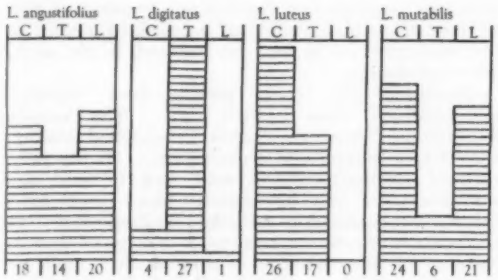

Fig. 1. Frequency of 3-nodule distribution attributes on four lupin species in effective symbiosis with nine *Rhizobium* strains

There was no detectable effect of *Rhizobium* strain in this experiment. Analysis shows that the three species *L. digitatus* ($\chi^2 = 24{\cdot}383^{***}$ on 2 d.f.), *L. luteus* ($\chi^2 = 37{\cdot}820^{***}$ on 2 d.f.) and *L. mutabilis* ($\chi^2 = 10{\cdot}941^{**}$ on 2 d.f.) exhibit preferential siting of nodules, but there is no evidence that *L. angustifolius* ($\chi^2 = 1{\cdot}079$ N.S. on 2 d.f.) does so.

Comparing patterns on the different species shows:

L. luteus	vs.	*L. angustifolius*	($\chi^2 = 21{\cdot}069^{***}$	on 2 d.f.)
,,	,,	*L. digitatus*	($\chi^2 = 18{\cdot}171^{***}$	,,)
,,	,,	*L. mutabilis*	($\chi^2 = 25{\cdot}864^{***}$	,,)
L. digitatus	,,	*L. angustifolius*	($\chi^2 = 26{\cdot}986^{***}$	,,)
,,	,,	*L. mutabilis*	($\chi^2 = 43{\cdot}792^{***}$	,,)
L. mutabilis	,,	*L. angustifolius*	($\chi^2 = 4{\cdot}065$ N.S.	,,)

These results show that legume species may exhibit preferential and differing nodulation patterns under uniform conditions, and that this may apply intragenerically on annuals of similar rooting habit.

We gratefully acknowledge the assistance of Mr. N. S. Stenhouse, statistician of this Institute.

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A Bivalve Gastropod

In the course of a collecting trip to Torquay, Victoria, in quest of marine molluscs, I had the great fortune to take alive that most peculiar bivalve, *Edenttellina typica* Gatliff and Gabriel. Much to my surprise I observed that the animal was that of a gastropod and apparently congeneric with the recently described *Tamanovalva limax* Kawaguti and Baba from Japan[1]. Even more surprising is the fact that I also collected, along with the above species, a second species of bivalved gastropod which although of the same group is undoubtedly of a different genus; shell and animal characters indicate many differences, but there is still a helicoid apex on the left valve only. Both species were collected on a species of the green seaweed *Caulerpa*, where it lives, as the Japanese authors indicate, among the roots.

The internal anatomy of both species has yet to be investigated, but it is hoped to publish preliminary descriptions as soon as possible.

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[1] See *Nature*, **185**, 749 (1960).

GENETICS

A Mosaic of *Drosophila* consisting of *1X*, *2X* and *3X* Tissue and its Probable Origin by Mitotic Non-disjunction

The renewed interest in abnormal distributions of chromosomes leads me to place on record an unusual mosaic individual of *Drosophila melanogaster* found in 1933 while working at the California Institute of Technology. A 'strange gynander' appeared among the offspring of a cross between a female which carried the dominant bristle gene Minute-*n* (*M-n*) on one *X*-chromosome and the recessive eye colour gene carnation (*car*) on the other, and a male which carried the recessive body colour gene yellow (*y*) on its *X*-chromosome.

No part of the gynander had Minute bristles. Its abdomen was of the female type. The whole right eye and the upper half of the left eye had carnation colour and the right foreleg possessed a sex-comb, characteristic for the male. The lower half of the left eye had normal eye colour but an abnormal, rough surface caused by irregular arrangement of the ommatidia. The left foreleg had no sex-comb. In contrast to the right wing, which was normal, the left wing was short and abnormal. The specimen was shown to C. B. Bridges, who pointed out that the roughness of part of the left eye and the abnormality of the left wing were traits characteristic of a super-female. The fly had had occasion to mate with its *car* brothers. It produced the following types and numbers of offspring: females, wild 35, *car* 36; males, *y* 20, *car* 20, wild 21, *y car* 8. Apparently the ovaries were of the genotype $+car/y+$.

In *Drosophila*, most gynanders are the result of elimination, during an early cleavage mitosis, of one of the two *X*-chromosomes of a female zygote[1]. In consequence, two unlike daughter cells are produced, one with a representative of each of the two *X*-chromosomes and another with only one *X*-chromosome. The mosaic recorded in this communication consisted of three types of cells, of the *X*-chromo-

animals. Sessile hydrozoans, polyzoans and sea anemones are attached to the surface while serpulids and sabellids live in tubes of their own buried within the tissues of the sponge with only their outer ends exposed. Asteroids, brittle stars, copepods, isopods and amphipods are seen to live in the canal systems of the sponges. Such animal associations are unusual in the case of the Royapuram sponges. Occasionally, however, a few colonies of hydrozoans or polyzoans are found growing on the surface of the sponges.

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by each of nine *Rhizobium* strains known to be effective in symbiosis with all species, in an experiment of factorial design: species (4) × strains (9) × replications (3). Plants were grown under controlled conditions in glasshouse sand-culture units. Four disinfected seeds were planted in each pot and inocula were applied to seeds in uniform quantities. Root systems were inspected after a period of plant growth of 50 days.

Three distinctive attributes of nodulation pattern were observed. Crown nodulation (*C*) was distinguished as any nodulation located on the tap root above the first lateral root. Tap-root nodulation (*T*) was distinguished as any nodulation on the tap root anywhere below the first lateral root. Lateral root nodulation (*L*) was distinguished as incidence of any nodules on lateral roots. Each attribute was not always represented in each replication of four plants. The frequencies of attribute incidence on the four species are given in Fig. 1, where the highest possible frequency is 27 (on the basis of 27 culture-unit replications).

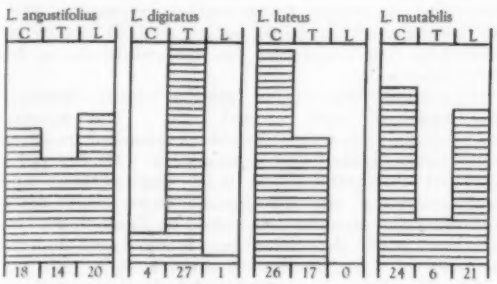

Fig. 1. Frequency of 3-nodule distribution attributes on four lupin species in effective symbiosis with nine *Rhizobium* strains

There was no detectable effect of *Rhizobium* strain in this experiment. Analysis shows that the three species *L. digitatus* ($\chi^2 = 24{\cdot}383^{***}$ on 2 d.f.), *L. luteus* ($\chi^2 = 37{\cdot}820^{***}$ on 2 d.f.) and *L. mutabilis* ($\chi^2 = 10{\cdot}941^{**}$ on 2 d.f.) exhibit preferential siting of nodules, but there is no evidence that *L. angustifolius* ($\chi^2 = 1{\cdot}079$ N.S. on 2 d.f.) does so.

Comparing patterns on the different species shows:

L. luteus	vs. *L. angustifolius*	($\chi^2 = 21{\cdot}069^{***}$	on 2 d.f.)
,,	,, *L. digitatus*	($\chi^2 = 18{\cdot}171^{***}$	,,)
,,	,, *L. mutabilis*	($\chi^2 = 25{\cdot}864^{***}$	,,)
L. digitatus	,, *L. angustifolius*	($\chi^2 = 26{\cdot}986^{***}$	,,)
,,	,, *L. mutabilis*	($\chi^2 = 43{\cdot}792^{***}$	,,)
L. mutabilis	,, *L. angustifolius*	($\chi^2 = 4{\cdot}065$ N.S.	,,)

These results show that legume species may exhibit preferential and differing nodulation patterns under uniform conditions, and that this may apply intragenerically on annuals of similar rooting habit.

We gratefully acknowledge the assistance of Mr. N. S. Stenhouse, statistician of this Institute.

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A Bivalve Gastropod

In the course of a collecting trip to Torquay, Victoria, in quest of marine molluscs, I had the great fortune to take alive that most peculiar bivalve, *Edenttellina typica* Gatliff and Gabriel. Much to my surprise I observed that the animal was that of a gastropod and apparently congeneric with the recently described *Tamanovalva limax* Kawaguti and Baba from Japan[1]. Even more surprising is the fact that I also collected, along with the above species, a second species of bivalved gastropod which although of the same group is undoubtedly of a different genus; shell and animal characters indicate many differences, but there is still a helicoid apex on the left valve only. Both species were collected on a species of the green seaweed *Caulerpa*, where it lives, as the Japanese authors indicate, among the roots.

The internal anatomy of both species has yet to be investigated, but it is hoped to publish preliminary descriptions as soon as possible.

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[1] See *Nature*, **185**, 749 (1960).

GENETICS

A Mosaic of *Drosophila* consisting of 1*X*, 2*X* and 3*X* Tissue and its Probable Origin by Mitotic Non-disjunction

The renewed interest in abnormal distributions of chromosomes leads me to place on record an unusual mosaic individual of *Drosophila melanogaster* found in 1933 while working at the California Institute of Technology. A 'strange gynander' appeared among the offspring of a cross between a female which carried the dominant bristle gene Minute-*n* (*M-n*) on one *X*-chromosome and the recessive eye colour gene carnation (*car*) on the other, and a male which carried the recessive body colour gene yellow (*y*) on its *X*-chromosome.

No part of the gynander had Minute bristles. Its abdomen was of the female type. The whole right eye and the upper half of the left eye had carnation colour and the right foreleg possessed a sex-comb, characteristic for the male. The lower half of the left eye had normal eye colour but an abnormal, rough surface caused by irregular arrangement of the ommatidia. The left foreleg had no sex-comb. In contrast to the right wing, which was normal, the left wing was short and abnormal. The specimen was shown to C. B. Bridges, who pointed out that the roughness of part of the left eye and the abnormality of the left wing were traits characteristic of a super-female. The fly had had occasion to mate with its *car* brothers. It produced the following types and numbers of offspring: females, wild 35, *car* 36; males, *y* 20, *car* 20, wild 21, *y car* 8. Apparently the ovaries were of the genotype $+car/y+$.

In *Drosophila*, most gynanders are the result of elimination, during an early cleavage mitosis, of one of the two *X*-chromosomes of a female zygote[1]. In consequence, two unlike daughter cells are produced, one with a representative of each of the two *X*-chromosomes and another with only one *X*-chromosome. The mosaic recorded in this communication consisted of three types of cells, of the *X*-chromo-

some constitutions *XX*, *X* and *XXX*. Its most likely origin was as follows. An *XX*-zygote of the genotype *+car/y+* underwent, in one of its early cleavage mitoses, a process of non-disjunction of the daughter chromosomes of the paternal *X*-chromosome which carried *y+*. Instead of moving to opposite poles, the two chromosomes were included in one daughter nucleus the three *X*-chromosomes of which carried the genotype *+car/y+/y+*, while the other daughter nucleus obtained a single *X*-chromosome only, with *+car*. The unchanged mitotic descendants of the *XX*-nuclei became the nuclei of at least the majority of the germ cells of the mosaic and probably of the cells of the whole abdomen and perhaps of parts of the thorax and head; mitotic descendants of the *XXX* nucleus became the nuclei of the cells of at least the lower half of the left eye and the left wing; and mitotic descendants of the *X*-nucleus became the nuclei of at least the right eye, the upper half of the left eye and the sex-comb-forming part of the right foreleg.

The origin of the mosaic could be explained in other ways than the one outlined here, but only by assuming a multiple sequence of abnormal processes[2]. The single event of mitotic non-disjunction proposed above accounts for the origin of the triple mosaic in a simple way.

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Dec. 22.

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Reversions induced by Base Analogues in *Salmonella typhimurium*

The base analogues, 5-bromouracil and 2-aminopurine, employed by Freese[1] to study the *r*II region of the genetic material of bacteriophage *T4* were also found to induce reversions from auxotrophy to prototrophy in *Salmonella typhimurium*. It was determined that 5-bromouracil can be incorporated into the deoxyribonucleic acid of this organism, replacing thymine specifically.

The tryptophan-*D* locus, known to block the last step in the biosynthesis of that amino-acid, was selected for these studies. The order of the nine mapped alleles within this locus has been established by transduction experiments and is the following[2]:

$$\text{Try-}D\ 1,\ 10,\ 35,\ 9,\ 6,\ 7,\ (55:69)\ \overset{66}{}$$

Each mutant strain was grown in the presence of the mutagen for about 10 divisions in minimal medium supplemented with 20 μgm. tryptophan/ml. and 0·2 per cent glucose. The concentrations of the mutagens were: 500 μgm. 2-aminopurine or 200 μgm. 5-bromouracil together with 200 μgm. aminopterin per ml. 5-Bromouracil was not effective in the absence of the folic acid inhibitor known to render the cells thymine-deficient. Following treatment the cells were washed and plated on minimal agar of two kinds: containing 0·01 per cent nutrient broth (enriched minimal) and without the enrichment.

The following results were obtained:

(*a*) Both base analogues increased the frequency of back mutation; from a spontaneous background of $0{\cdot}5\text{–}1{\cdot}0 \times 10^{-8}$, it was raised to $5\text{–}15 \times 10^{-8}$ in the presence of 5-bromouracil, and to $5\text{–}10 \times 10^{-7}$ with 2-aminopurine. The number of revertants could also be increased by plating untreated cells directly on enriched minimal agar containing 5-bromouracil and aminopterin.

(*b*) The increase in the number of recovered prototrophs was due to base analogue-induced mutations and not to selection of spontaneous revertants present during growth in the liquid medium. This was established in two ways: (1) by reconstruction experiments, where a tryptophan requirer was mixed with wild-type cells in known ratios; (2) by the fact that a significantly higher number of revertants was always recovered when the bacteria were plated on enriched minimal than on unsupplemented minimal agar.

(*c*) There was a differential response of the alleles to the mutagen. All except tryptophan *D*-9 and -55 reverted to prototrophy with different frequencies in the presence of the base analogues (see Balbinder[2]). With the exception of the mutant tryptophan *D*-6, all mutants which could be induced to revert by 2-aminopurine could also be induced to do so by 5-bromouracil.

Correlated with these genetic data, chemical investigations were carried out. The mutant tryptophan *C*-3 was cultured in enriched medium with 5-bromouracil and aminopterin. Highly polymerized deoxyribonucleic acid was isolated and estimations of the nitrogenous bases were made following the procedure described by Zamenhof[3]. On the basis of two determinations, it was found that the amount of 5-bromouracil was 30·0–33·2 mole per cent of thymine. (5BrU/5BrU + T = per cent incorporation of 5-bromouracil.) Data for the incorporation of 2-aminopurine are not available.

Freese[4] postulated that mutations of this kind are not caused merely by the replacement of a deoxyribonucleic acid base by its analogue but through mistakes in base pairing which the analogue may undergo at some constant frequency. Thus once the base analogue has been incorporated, the change in the site is dependent solely upon subsequent deoxyribonucleic acid replication. Our results tend to support this hypothesis since more revertants were recovered upon plating on enriched minimal agar, where the cells undergo a number of divisions, than on unsupplemented minimal agar.

Although the number of mutants analysed is small, the results thus far parallel those obtained by Freese. We have demonstrated that the tryptophan *D* locus contains base analogue-inducible and non-inducible alleles.

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Genetics of Resistance to Dieldrin in *Lucilia cuprina* Wied.

In an earlier study[1] on the inheritance of dieldrin resistance in *Lucilia cuprina*, involving a cross between susceptible females and a single resistant male, *F*2 and back-cross ratios, obtained by employing the discriminating dose technique[2,3], indicated that dieldrin resistance in this insect can be explained in terms of a single gene. Information is now presented from reciprocal crosses between resistant and susceptible strains. The discriminating dose method was again utilized, with the results shown in Tables 1 and 2. The data from the *F*2 and back-cross ratios suggest a simple Mendelian interpretation, even though the *F*1 and *F*2 data conform to a polygenic pattern, namely, intermediate resistance in *F*1 progeny with both parental types in the *F*2.

The following reciprocal crosses were made: (*a*) Single pair mating, resistant female (virgin female, which survived 4 μgm. of dieldrin) × a susceptible male, (*b*) Semi-mass cross of 10 susceptible females × 5 resistant males (males survived 2 μgm. of dieldrin per fly).

The *F*1 were mass interbred for the *F*2 generations. Each back-cross was made with 10 virgin females × 5 males. The dieldrin-resistant parents for the back-crosses were survivors of the respective high discriminating doses.

As a basis for the investigation, low and high discriminating doses were determined for both the susceptible and *F*1 male and female flies, respectively, by the topical application of dieldrin in kerosene to the thorax of the flies by a method described previously[1]. These doses are given as a footnote to Table 1, and it will be observed that the discriminating doses for females are twice the levels for males.

It is important to record that reversion towards susceptibility has been observed with selected resistant strains of *Lucilia cuprina* currently being cultured in the laboratory for genetical studies. Full reversion to susceptibility has been observed in 25 generations. An approximate 1 : 1 ratio (42 per cent mortality, 100 female flies tested) occurred when a back-cross was made between virgin females from a reverted strain with *F*1 males (susceptible females × resistant males).

Table 1. 24-HR. MORTALITIES PER CENT OF *F*1 AND *F*2 PROGENY OF RECIPROCAL CROSSES BETWEEN RESISTANT AND SUSCEPTIBLE *Lucilia cuprina* AT DISCRIMINATING DOSAGE-LEVELS

	Sex	Low discriminating doses*	No. tested	High discriminating doses†	No. tested
Resistant female × susceptible male					
*F*1	♀	2	100	100	100
	♂	6	100	100	100
*F*2	♀	24·5	200	71	100
	♂	24	200	80	100
Susceptible females × resistant males					
*F*1	♀	0	100	100	100
	♂	8	150	100	100
*F*2	♀	25	100	82	100
	♂	24·5	200	81	100

* Low discriminating doses: females, 0·1 μgm. per fly; males, 0·05 μgm. per fly.
† High discriminating doses: females, 4 μgm. per fly; males, 2 μgm. per fly.

Table 2. 24-HR. MORTALITIES PER CENT OF BACK CROSS *Lucilia cuprina* PROGENY AT DISCRIMINATING DOSAGE-LEVELS

	Sex	Low discriminating dosage*	No. tested	High discriminating dosage†	No. tested
Resistant female × susceptible male					
Back-crosses					
*F*1 ♀ × resistant ♂	♀	3	100	51	100
	♂	3	100	58	100
*F*1 ♂ × resistant ♀	♀	0	75	48·1	96
	♂	2·1	96	49	100
*F*1 ♀ × susceptible ♂	♀	50	100	100	100
	♂	52·7	150	100	90
*F*1 ♂ × susceptible ♀	♀	53·5	200	100	100
	♂	51	100	100	100
Susceptible females × resistant males					
Back-crosses					
*F*1 ♀ × susceptible ♂	♀	46	100	100	100
	♂	51	100	100	100
*F*1 ♂ × susceptible ♀	♀	51	100	100	100
	♂	52	144	100	100
*F*1 ♀ × resistant ♂	♂	0	100	48·1	96
	♀	2	100	53	100
*F*1 ♂ × resistant ♀	♀	1·3	150	50	242
	♂	1	100	44	100

* See footnote to Table 1.

Grateful acknowledgment is made to the Commonwealth Wool Committee for providing Research Trust Funds for this inquiry.

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PSYCHOLOGY

Dichotic Stimulation and Memory Disorder

The responses made by young normal adults to various methods of dichotic stimulation have been extensively investigated and reported upon by Broadbent[1]. His observations have led him to postulate that both perceptual ('*p*-system') and storage ('*s*-system') mechanisms are necessary for the production of correct responses to such stimulation. If this were the case, then the transmission of such material should be disrupted by any disturbance of the storage system. I have suggested[2] that a gross form of such disturbance is to be found in elderly persons suffering from memory disorder.

The following report is intended to provide advance information on a study designed to investigate the effect of memory disorder on responses to different stimuli delivered to both ears at once. It is hoped that the results to be reported will facilitate clinical research into this technique, which may come to provide a simple, reliable method for the early detection of 'storage breakdown'.

Two groups of subjects were tested. Group *A* consisted of 15 elderly patients aged 68–83, known to be suffering from memory disorder. Group *B* was similarly composed of 15 patients, aged 66–84, in whom no memory disorder was suspected. These two groups were matched in terms of their ability to repeat a digit span series orthodoxly delivered.

The dichotic series consisted in four spans of each length from 1 to 4 digits recorded in each channel of a

Table 1

Length of half-span	Dominant		Non-dominant	
	Group *A*	Group *B*	Group *A*	Group B
1	0·98	1·00	0·47	0·95
2	1·83	1·95	0·09	1·80
3	2·42	2·90	0·05	2·15
4	2·82	3·63	0·09	0·95

'Webcor' stereophonic tape recorder. These were played back to the subject through Edison (Model 30) earphones.

Table 1 presents the average number of correct responses made to both the 'dominant' (that is, the first half-span recalled) and the 'non-dominant' (that is, the second half-span recalled) series by both groups.

In accordance with Broadbent's suggestion that the second half series recalled is typically the one which has passed through the *s*-system it can be seen from the results that there is a significant difference ($P < 0{\cdot}01$) between the lengths of the non-dominant spans recalled by the two groups. The average score made by group *A* is in each case lower than that made by group *B*.

If the present finding is confirmed by further research, it appears that while the orthodox digit span is the same for both groups, dichotic spans are clearly different. This method may permit the rapid, early detection of impairment of the storage process.

The research outlined above was made possible by a grant from the Arts Research Committee, Queen's University, Kingston. The testing was carried out by Mr. R. E. Sanderson.

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Construct 'Reactive Inhibition' in the Interpretation of Age Changes in Performance

We have become interested in the possibility of extending the theory of ageing by interpreting age changes in performance within the framework of general behaviour theory. It seems to us that the Hullian concept of 'reactive inhibition'[1] might be particularly useful in this respect in that the assumption that reactive inhibition is generated more rapidly and dissipated more slowly as the age of an organism increases is compatible with many of the accepted changes which occur with age, and leads to predictions regarding changes which as yet have not been observed.

If this assumption is found to be valid, then older organisms may have to be regarded, after Eysenck[2], as more extraverted than younger ones. This definition of one aspect of personality of older organisms, if it is found to be valid, may be useful in the general study of personality changes which occur with age, a field of study which, in the past, has been somewhat lacking in a theoretical basis which encourages experimental investigation. Broadbent[3] has discussed the existing evidence that older persons are more or less extraverted than younger ones and, while concluding that the evidence is inadequate and that no valid inference may as yet be drawn, he appears to favour the idea that they become, in fact, more introverted. Braun and Geiselhart[4] report that it is more difficult to establish conditioned responses as age increases. Although these authors do not interpret their results in the way we have suggested, it is possible that the more rapid generation of reactive inhibition may be responsible for the behaviour of older subjects which they observe. The existing evidence is incomplete. The issue appears to be a real one and to merit further experimental investigation.

In order to test our hypothesis more directly we have studied the responses of forty university students between the ages of 18 and 25 years and of twenty-five retired professional men and women between the ages of 63 and 86 years in reporting the after-effect of movement seen after fixating the centre of a rotating Archimedes spiral. A short after-effect is associated, according to Eysenck, with extraversion, and the theoretical explanation put forward to account for this is as follows: the stimulation arising from fixation generates reactive inhibition as well as the excitatory state it sets up simultaneously, and the length of the after-effect is reduced partly by the amount of inhibition generated during fixation and partly by the amount generated during the after-effect itself. The test is regarded as being a good index of extraversion, and short after-effects have been demonstrated to be characteristic of hysterics in contrast to dysthymics (Eysenck, H. J., personal communication), and to be induced by depressant (so called 'extraverting') drugs[2].

In our experiment all subjects fixated the centre of a four-throw spiral drawn in black on a white card of 10 in. diameter which revolved at 78 r.p.m. At the end of 40 sec. the rotation ceased and subjects reported when the after-effect could no longer be observed. The spiral was then again rotated, this time for 30 sec. and at its cessation an accurate record was made of the duration of the after-effect. The mean duration of after-effect after the second rotation was 10·9 sec. in the case of younger subjects ($S.D. = 6{\cdot}3$ sec.), and 4·0 sec. in the case of older subjects ($S.D. = 4{\cdot}3$ sec.). The value of t associated with the difference between these means is a little higher than 5·2, demonstrating the observed difference to be very significant indeed. This result, we believe, supports the hypothesis that reactive inhibition generates more rapidly in older organisms than in the young.

Further predictions from the hypothesis are being tested: these include predictions that older subjects will show a greater decrement than younger ones in the performance of vigilance tasks, and that older subjects will show greater reminiscence after massed practice than younger ones. Consideration is also being given to the possible adaptive function of the apparently greater rapidity with which reactive inhibition is generated by older organisms as compared with younger ones.

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FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Monday, April 11

MEDICAL RESEARCH COUNCIL (at the National Institute for Medical Research, Mill Hill, London, N.W.7), at 5 p.m.—Dr. F. Jacob (Institut Pasteur, Paris): "Genetic Control of Regulation Mechanisms in Bacteria".*

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Public Lighting" opened by Mr. A. E. J. Whitcher.

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Annual General Meeting, followed by Dr. E. E. Turtle: "Trends in the Control of Infestation of Stored Products".

MANCHESTER LITERARY AND PHILOSOPHICAL SOCIETY (in the Reynolds Hall, Manchester College of Science and Technology, Sackville Street, Manchester), at 5.45 p.m.—Dr. G. B. B. M. Sutherland, F.R.S.: "British and American Universities—Some Comparisons".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. Anthony Forge: "Art and Society in New Guinea".

Tuesday, April 12

POWDER METALLURGY JOINT GROUP of the IRON AND STEEL INSTITUTE and the INSTITUTE OF METALS (in the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 10.30 a.m.—Informal Discussion on "Direct Rolling Processes in Powder Metallurgy".

ZOOLOGICAL SOCIETY OF LONDON (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Electronic Aids for the Blind", opened by Lord Fraser of Lonsdale, C.H., C.B.E., and Dr. R. L. Beurle.

Wednesday, April 13

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. C. L. C. Allan: "Water-Turbine-Driven Induction Generators".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Annual General Meeting, followed by Dr. F. Aylward: "Nutrition in Relation to the Biological and Social Sciences" (Chairman's Address).

BRITISH INSTITUTION OF RADIO ENGINEERS, LONDON SECTION (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. F. R. J. Spearman: "Guided Weapon Control".

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Mr. J. A. Loraine: "The Assay of Gonadotrophins in Body Fluids"; Mr. P. H. Wright: "Insulin Assay in Plasma by the Rat Diaphragm Method".

WOMEN'S ENGINEERING SOCIETY (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7 p.m.—Dr. L. P. Smith: "Plastics in Engineering".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER IN MATHEMATICS; and an ASSISTANT, Grade "B", IN CHEMISTRY, mainly inorganic chemistry, at Birkenhead Technical College—The Director of Education, Birkenhead Education Committee, 63 Hamilton Square, Birkenhead (April 13).

RESEARCH ASSOCIATE (graduate, with special qualifications in physical chemistry, physics or mineralogy and petrology) IN THE DEPARTMENT OF GEOLOGY, for work upon hydrated alumino-silicate glasses and melts and for the development of apparatus for such work—Prof. F. W. Shotton, University of Birmingham, Edgbaston, Birmingham 15 (April 19).

LECTURER IN APPLIED MATHEMATICS (Digital Computing)—The Secretary, The Queen's University, Belfast (April 20).

LECTURER or ASSISTANT LECTURER (qualified in pure or applied mathematics) IN MATHEMATICS—The Registrar, University College of North Staffordshire, Keele, Staffs (April 21).

ASSISTANT LECTURER (with special interests in structural geology or in stratigraphical palæontology) IN THE DEPARTMENT OF GEOLOGY—The Registrar, The University, Manchester 13 (April 23).

DEMONSTRATOR IN THE DEPARTMENT OF PHYSICS—The Registrar, King's College (University of Durham), Newcastle upon Tyne 1 (April 23).

SENIOR LIBRARIAN (Chartered Librarian or with equivalent qualifications)—Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (April 25).

CHAIR OF AGRICULTURE in the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 27).

ESSO STUDENT (with, or expecting to hold, an honours degree in chemistry or engineering) IN CHEMICAL ENGINEERING—The Registrar, Imperial College of Science and Technology, London, S.W.7 (April 29).

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree or five subjects at G.C.E. "O" level and at least two "A" level) IN THE DEPARTMENT OF GENETICS, to assist in the fields of biochemical and fungal genetics—The Secretary, John Innes Horticultural Institution, Bayfordbury, Hertford, Herts (April 30).

ASSISTANT IN THE DEPARTMENT OF CHEMISTRY—The Secretary, The University, Edinburgh (April 30).

ASSISTANT LECTURER IN PHILOSOPHY—The Registrar, The University, Manchester (April 30).

ASSISTANT LECTURER or LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Sheffield (April 30).

CHIEF or SENIOR TECHNICIAN (preferably with experience in biological work) IN THE PHYSIOLOGY DEPARTMENT—The Secretary, Marischal College (University of Aberdeen), Aberdeen (April 30).

LECTURER IN INORGANIC CHEMISTRY—The Registrar, The University, Manchester 13 (April 30).

CURATOR (with a university degree and experience of museum work) of the King George V Memorial Museum—The Secretary to the Trustees, King George V Memorial Museum, Box 511, Dar-es-Salaam (May 1).

LECTURER IN BIOCHEMISTRY; an ASSISTANT LECTURER IN AGRICULTURAL CHEMISTRY; and an ASSISTANT LECTURER IN GEOLOGY—The Registrar, Wye College (University of London), near Ashford, Kent (May 1).

ASSISTANT (with an honours degree in biochemistry, agricultural biochemistry or equivalent, and preferably an interest in endocrinology) IN AGRICULTURAL BIOCHEMISTRY—The Secretary, The University, Aberdeen (May 2).

LYONS POSTGRADUATE STUDENT (with an honours degree in chemistry from a British university), for research or postgraduate study in chemistry, particularly physical or organic chemistry—The Registrar, Imperial College of Science and Technology, London, S.W.7 (May 2).

LECTURER IN PURE MATHEMATICS and a LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Hull (May 4).

LECTURER IN BOTANY (MYCOLOGY)—The Secretary, The University, Aberdeen (May 5).

ASSISTANT LECTURER or LECTURER (preferably with experience in the use of a mass spectrometer) IN THE DEPARTMENT OF GEOLOGY, to specialize in radiogeology and preferably to work on geological age determinations—The Registrar, The University, Liverpool, quoting Ref. CV/T (May 7).

LECTURER IN MINERALOGY IN THE DEPARTMENT OF GEOLOGY—The Secretary, The University, Edinburgh (May 7).

READER (with special interests and qualifications in the experimental study of the cognitive processes) IN PSYCHOLOGY at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, May 7).

UNIVERSITY STUDENTS and FELLOWS, for experimental or theoretical research in solid state physics—The Professor of Natural Philosophy, The University, Glasgow (May 7).

READER IN VETERINARY BIOCHEMISTRY at the Royal Veterinary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (May 9).

ASSISTANT LECTURERS or LECTURERS (with special interests in marine, vertebrate or invertebrate zoology or protozoology) IN ZOOLOGY at the University College of Ghana—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 10).

FELLOW IN THE CHEMISTRY DEPARTMENT, for research on the flow of gases in fine capillaries—Prof. R. M. Barrer, F.R.S., Chemistry Department, Imperial College of Science and Technology, London, S.W.7 (May 14).

LECTURER IN PHYSICAL CHEMISTRY—Prof. R. M. Barrer, F.R.S., Imperial College of Science and Technology, London, S.W.7 (May 14).

SCIENTIFIC OFFICER (with an appropriate first- or second-class honours degree, and preferably a knowledge or experience of plant breeding), for work on vegetable crops; and an ASSISTANT EXPERIMENTAL OFFICER (with a pass degree in botany with chemistry and zoology, and preferably some knowledge of statistics), to assist with work on genetics of horticultural crops, particularly routine chromosome counting and field recording—The Secretary, Scottish Horticultural Research Institute, Mylnefield, Invergowrie, by Dundee (May 15).

SENIOR LECTURER and a LECTURER IN MATHEMATICS at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 20).

RESEARCH FELLOW (organic chemist) IN THE DEPARTMENT OF MEDICAL CHEMISTRY, John Curtin School of Medical Research, Australian National University, Canberra, to work with Prof. Adrien Albert or Dr. D. J. Brown on problems in the pyrimidines, pteridines or other hetero-aromatic series—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 31).

ASSISTANT, Grade B (graduate, preferably possessing a special degree in geology with ancillary botany and zoology), IN THE NATURAL SCIENCE DEPARTMENT, to teach geology and biological subjects—The Principal, Derby and District College of Technology, Kedleston Road, Derby.

ASSISTANT PROFESSOR (preferably Ph.D. or equivalent) OF CHEMICAL ENGINEERING, for duties which include graduate and undergraduate instruction and direction of graduate research—Head, Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

BIOCHEMICAL ASSISTANT, to assist with diabetic research—The Director, Medical Unit, King's College Hospital Medical School, Denmark Hill, London, S.E.5.

BIOCHEMIST (previous hospital experience an advantage but not essential) FOR THE PATHOLOGICAL LABORATORY—The Secretary, Highlands General Hospital, Winchmore Hill, London, N.21.

LECTURER (with a good honours degree in physics, with teaching and/or research experience) IN PHYSICS—The Clerk to the Governors,

South-East Essex Technical College and School of Art, Longbridge Road, Dagenham, Essex.

Lecturer (with expert knowledge and experience of the practical application in one or more of the fields of elasticity, plasticity and vibrations of solid bodies) in Applied Mechanics in the Department of Aircraft Design—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

Lloyd-Muirhead Fellow in Social Philosophy—The Assistant Registrar, The University, Edmund Street, Birmingham 3.

Physicist, Metal Physicist or Physical Metallurgist (with a good honours degree and the ability to plan and interpret experiments), to carry out fundamental research aimed at providing a theoretical understanding of the mechanisms which cause changes in fuel materials when under irradiation—The Group Recruitment Officer (1599/34), U.K.A.E.A., A.E.R.E., Harwell, Didcot, Berks.

Post-Doctoral Fellow, for fundamental research on the biosynthesis and interaction with proteins of cereal starches—The Deputy Registrar, The University, Birmingham 15.

Research Fellow (university graduate with a good research background, and preferably with a higher degree, or intending to work for one) in Polymer Science in the Department of Materials—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

Scientific Officer or Senior Scientific Officer (with an honours degree in physics with an interest in the application of physical/chemical procedures to macromolecular systems) in the Biophysics Department, to undertake studies on the structure of viruses, on virus-antibody interaction and on physical problems relating to dissemination of viruses—The Secretary, Research Institute (Animal Virus Diseases), Pirbright, Surrey.

Senior Lecturer (qualified to direct research and to teach to honours degree or Diploma in Technology levels, and preferably industrial experience in a responsible position) in Textile Physics—The Registrar, Institute of Technology, Bradford 7.

Senior Technical Officer (graduate, with experience of experimentation with British agricultural crops, and preferably some experience of grassland husbandry), to conduct trials and tests on legumes and grasses; and an Assistant Systematic Botanist (graduate in botany or agricultural botany)—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

Sixth Form Physics Master—The Headmaster, Bolton School, Bolton, Lancs.

Technician (A.I.M.L.T. or equivalent) for the Routine Section of the Department of Chemical Pathology—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

Technician, to assist in work on medical applications of radioactive isotopes—The Superintendent, Guy's Hospital, London Bridge, London, S.E.1.

Technician (with some experience in biochemistry and haematology) in the Pathological Laboratory, Wanstead Hospital, Hermon Hill, London, E.11—The Secretary, Forest Group H.M.C., Langthorne Road, London, E.11.

University Demonstrator in Biochemistry—The Administrator, Department of Biochemistry, The University, South Parks Road, Oxford.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Russian Review of Biology, Vol. 48, No. 1 (July–August, 1959). Translated from *Uspekhi Sovremennoi Biologii*. Pp. 1–110. Published six times a year: issue for issue following the Russian edition. Subscription: 180*s.* or 30 dollars for the first nine issues (Vol. 48, parts 1, 2 and 3 of 1959, and six issues of 1960.) Thereafter annual subscription will be 130*s.* or 20 dollars. Single issues 30*s.* or 5 dollars. (London and Edinburgh: Oliver and Boyd, Ltd., 1959.) [261

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1005, Vol. 252 (21 January 1960): Matrix and other Direct Methods for the Solution of Systems of Linear Difference Equations. By W. G. Bickley and J. McNamee. Pp. 69–131. 20*s.* No. 1006, Vol. 252 (21 January 1960): An Experimental Study of the Enthalpy of Steam. By G. S. Callendar and Sir Alfred Egerton, F.R.S. Pp. 133–164. 10*s.* (London: Royal Society, 1960.) [261

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Carnegie Institution of Washington Year Book No. 58, July 1, 1958—June 30, 1959. Pp. xi+500+15 plates. (Washington, D.C.: Carnegie Institution of Washington, 1959.) 1 dollar, paper bound; 1.50 dollars, cloth bound. [261

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